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One Health Triad

Editors:
Rao Zahid Abbas, Nahla M Saeed, Muhammad Younus,
Liliana Aguilar-Marcelino and Ahrar Khan

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ONE HEALTH TRIAD
Volume 2

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EDITORS

VOLUME 2

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PREFACE

The well-being of humans and animals is pretty much interdependent. It’s impossible to ensure human health, food security and food safety, and welfare without considering animal and environmental health.

The need to enhance the collaboration between animal health workers and medical professionals, researchers and academicians has moved the editors to develop this publication. The book takes into account the major threats of animal, human and environmental health. This book provides the core concepts of One Health approach with a critical focus on the key challenges i.e., zoonotic diseases and environmental degradation. The objective is to cover epidemiological interactions of various infectious diseases and their environmental and ecological implications as an emerging threat.

It is anticipated that this book would be of great use to a variety of readers. University students, graduates, practitioners, animal healthcare providers and health professionals would definitely find this book of great importance. The language of book has been intentionally kept easier for a non-technical person to grasp the concepts on interdependence of animal, human and environmental health. The editors wish to publish a series on the subject keeping in view the urgency to highlight these areas for awareness, research and development.

Editors

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1

Antimicrobial Residues and Food of Animal Origin

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INTRODUCTION

Antimicrobial compounds produced naturally by microorganisms, plants, or modified chemical substances, and are used extensively for the treatment or cure of various infections in humans and animals (Asredie and Engdaw 2015). Many antimicrobial products are used in food-producing animals for different purposes i.e. to treat disease, as feed additives, and to enhance animal growth. The widespread consumption of these products results in antimicrobial residues in the tissues of these animals and the foods derived from them. On the other hand, these residues have numerous consequences on health and on a broad perceptive, on the environs (Treiber and Beranek-Knauer 2021). Fig. 1 illustrate the antibiotics given to animals through feed, water, injections as a feed additive, as growth promoter, and for treatment of disease, lead to antibiotic residues in food animals.

Metabolites called antimicrobial residues are formed after antimicrobial chemicals are administered to an animal and can be found in both non-edible and edible tissues. These antimicrobial residues in animal food that exceed the maximum acceptable limit may cause antibiotic resistance and other complications in humans and animals (Ngangom et al. 2019).

Antimicrobial Products used for Growth Promotion

Antimicrobial compounds are used to boost animal growth, primarily in fattening animals or broilers. The reason for the better growth is due to the gut's mucosal membrane becomes thinner, enabling improved absorbance, changing gut mobility to improve more suitable absorption and making optimal conditions for valuable microorganisms in the animal's gut by killing pathogenic bacteria. Furthermore, antimicrobials promote growth by reducing the immune system's scope of action, reducing toxin generation, and reducing nutrient waste. Antimicrobials used for growth promotion are particularly effective in poultry and small growing animals since they are more receptive to them. According to research, chicks fed the fermenting waste of manufactured tetracycline as a source of vitamin B_{12} matured quicker than hens fed as the controls. It was soon determined that the tetracycline residue (not the vitamin level in the feed) was the reason of this influence (Edqvist and Pedersen 2001).

Antimicrobial Products used for Therapeutics

The use of antimicrobials in all cases of inflammation, fever, viral infections and injuries has a significant residual effect on edible tissues. Antimicrobials are effective in usual conditions since their job is to eliminate frequently diverging cells through cell invasion (Mahmoudi et al. 2018).

Antimicrobial Products used for Prophylactics

Poultry or animals have been administered suboptimal groups of antimicrobials to stop potential illness. However, the antimicrobial agents are explicit in the range of their action merely in the vigorous reproducing phase of microorganisms; nevertheless, they will not deliver complete defense. In specific cases, i.e., surgical methods and dry cow therapy need antibiotic treatment (Callens et al. 2012).

Other use of Antimicrobial Products

These antimicrobial products are also utilized in many practices throughout the processing, manufacturing, and storing of dairy products. The contaminants in dairy products may arise from water, soil, or air through manufacturing, transportation, or storage. Moreover, the indirect contamination through feed provided to animals leads to antimicrobial residues in man. Various other major issues include the deficiency of extension activities, shortage of safe drugs, insufficient information given by manufacturers, utilization of animals for additional profit or production and unawareness. The United States Food and Drug Administration bans the additional usage of furazolidone,

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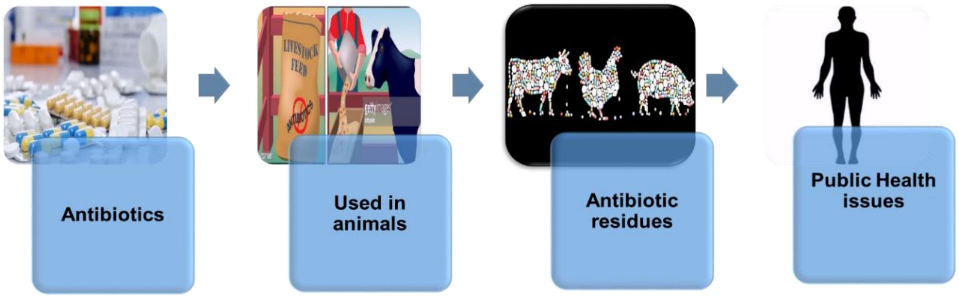

Fig. 1: Development of antibiotic residues.

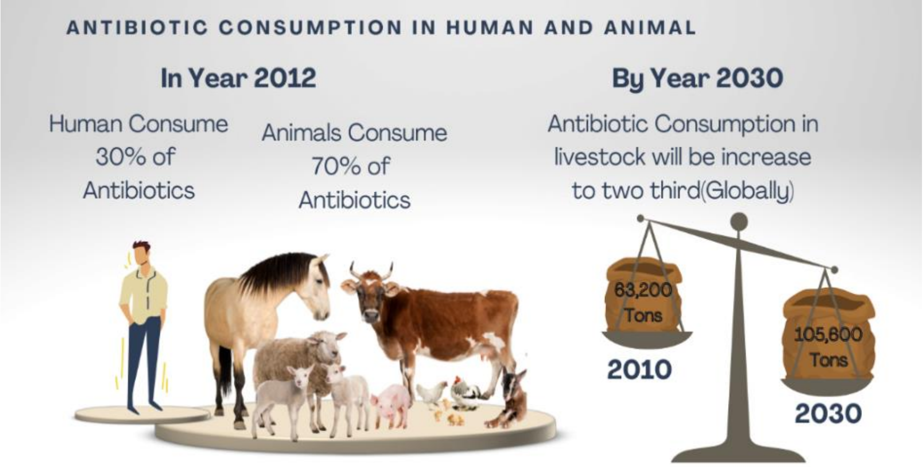
ANTIBIOTIC CONSUMPTION IN HUMAN AND ANIMAL
In Year 2012
Human Consume 30% of Antibiotics
Animals Consume 70% of Antibiotics
By Year 2030
Antibiotic Consumption in livestock will be increase to two third(Globally)
63,200 Tons
2010
105,600 Tons
2030

Fig. 2: Global consumption of antibiotics in humans and animals.

chloramphenicol, sulphonamide, fluoroquinolones, and nitrofurazone in lactating animals (Nisha 2008).

Widespread Antimicrobial Gourmandization in Food of Animals Origin

In 2010, there were roughly 63,151 (or 1,560) tonnes of antimicrobials used in animals raised for food. By 2030, it is predicted that this quantity will rise by 67% to 105,596 (or 3,605) tonnes. According to estimates, the rise in the number of animals bred for food production accounts for two-thirds (66%) of the overall increase (67%) in the consumption of antibiotics (Tiseo et al. 2020). While the farming methods used to produce remaining of the third proportion (34%) have changed, with a bigger proportion of animals by 2030 anticipated to be grown in intense agricultural setups. Due to the shift in production systems, Asia alone is consuming around 46% of antimicrobial products by 2030. Asia is predicted to consume 51,851 tonnes per annum of antimicrobials in 2030, which is 82% of the present worldwide consumption in 2010 in food animals (Van Boeckel et al. 2015). Fig. 2 highlights the global consumption of antibiotics in humans and animals.

According to WHO, today almost eighty percent of food-producing animals have been given different drugs in most or part of their life. Frequently used antimicrobials include sulphonamides, aminoglycosides, tetracyclines, spectinomycin, trimethoprim, macrolides, nitrofurans, Nitroimidazoles, β-lactams, pleuromutilins, Lincosamides, polymyxins, and quinolones (Cháfer-Pericás et al. 2010; Tadesse and Tadesse 2017).

The topmost antibiotics used in cattle include macrolides and tetracycline through drinking water and feed while sulphonamides as a therapeutic and prophylactic drugs and growth promoters. Generally, tetracycline, florfenicol and tylosin are used orally for the treatment of diarrhea, respiratory diseases, foot rot, and liver abscesses at sub-therapeutic levels (Mor et al. 2012). Fig. 3 represent the percentage of antibiotics to be used in animals.

Maximum Residue Limit

The maximum residue limit (MRL) is a concentration of antimicrobial products below the hazardous level and permitted by various organizations in feed or food projected to be used for human and animal intake at a given duration called as maximum residue limit. The measuring unit of MRL for liquid is mg/L and for solid mg/kg (Sachi et al. 2019). The food product below the MRL is non-hazardous for the health of consumers and does not interrupt manufacturing procedures. The concept of MRL maintains the balance between producer and consumer to use antibiotics safely. The main points to be considered while calculating MRL include potential effects or the toxicological possibility of residues on the digestive flora of humans (Anonymous, 2009; Ma et al. 2021).

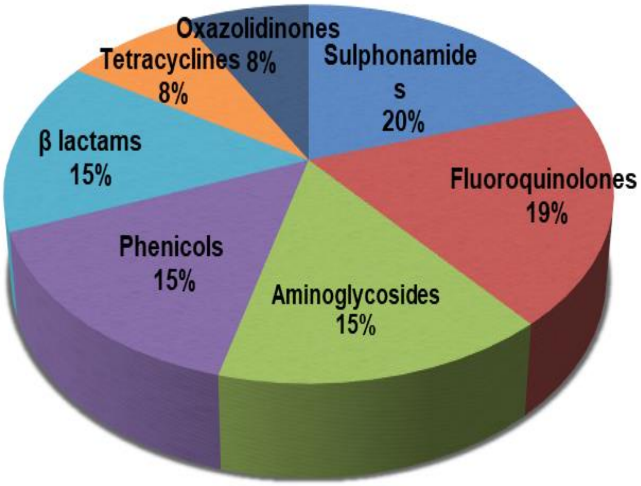

Fig. 3: Percentage of antibiotics used in animals.

Withdrawal Time

The time interval required after the administration of the drug in food-producing animals to ensure that the toxicological impact of antibiotic residue reaches a safe concentration or below MRL in milk or edible portion of the animal. This time reduces or prevents the detrimental effects of drug residues in foodstuffs for consumption (Ngangom et al. 2019). This largely depends on the route of administration, and the physical and chemical properties of drugs (Sachi et al. 2019). For checking antibiotic residues, throughout withdrawal procedures after drug administration is being stopped, the targeted tissue is selected and animal samples are collected and tested at different time intervals. Furthermore, the kidney and liver are the most important organs as residues required a longer time to pass through these organs (Ngangom et al. 2019).

Causes of Antibiotic Residues

The main causes of antibiotic residues in edible portions or tissue comprise overdose of injection, negligence to follow proper withdrawal time, use of contaminated equipment with antibiotics while administrating or mixing the drug, error in mixing and accidental feeding with medicated feed or spilled chemicals (Chen et al. 2019). Among the different routes of administration i.e. topical, oral and parenteral, it has been described that antimicrobial residues surpassing the criteria are mainly faced when directed through injection. This causes persistence in animal tissue, limiting their elimination (Arsène et al. 2022). Animal factors include pregnancy, allergies, age, infections, congenital illness, etc. Temperature variation of water for aquatic species, contaminated environment, and improper use of antibiotics. The presence of antimicrobial residues in seafood might result in the development of toxicity and bacterial resistance to customers, which can result in various diseases and even can cause disease in severe case. (Okocha et al. 2018).

The global requirement for products of animal is increasing as it is rich in protein for human consumption, commonly leading to the excessive utilization of antimicrobials in agriculture. Antimicrobials are used in 73 percent of meat production around the world. Assessments on the use of antimicrobials concerning the manufacturing of products of animal are expected to increase in 2030 by 11.5%. The spontaneous emergence of antimicrobial resistance in farm animals and indirectly in humans through animal food consumption is mainly due to the addition of antimicrobial compounds in animal feed. Apart from this issue the use of wastewater contaminated with antibiotics and dung disposal of animals in the environment are additional challenges that are repeatedly revealed in many scientific studies as a source of the spread of antibiotic residue and antimicrobial resistance (Treiber and Beranek-Knauer 2021). Fig. 4 highlights the major sources of antibiotic residues.

Public Health Risk

Public health risks arise by ingesting food containing antimicrobial residue above the acceptable daily intake or residual limit higher than MRLs. The sudden consequence of antimicrobial residue induces various illnesses throughout the food chain in humans. The adverse effects involve exposure to antimicrobial residues for a relatively long time (Agmas and Adugna 2018). Dairy and other food products from animals that contain antibiotic residues have major health issues as listed in Fig. 5. The harmful impact of the drugs comprises immunological diseases, cancer, toxic effects, (e.g. oxytetracycline, sulphamethazine, and furazolidone), nephropathy, mutagenicity, (e.g., gentamicin), and bone marrow toxicities, reproductive disorders, hepatotoxicity (e.g., chloramphenicol) (Tadesse and Tadesse 2017).

The possible effects of antimicrobial residues on public health are as follows:

- **Antibiotic Resistance:** The development of antibiotic resistance due to these residues is a major concern as these promote resistant bacteria and induce severe pathologies. Even the existence of low antibiotic residues renders microbes resistant to antibiotics. The antibiotic-resistant microorganisms can be transferred between people through contact directly or indirectly via exchanging resistant genes in the surroundings, further details of antimicrobial resistance are discussed below in the chapter.
- **Allergic Reactions:** Numerous allergic responses, such as anaphylaxis and serum sicknesses, are linked to various antibiotic residues, primarily in penicillin's case.
- **Carcinogenicity:** Antibiotic residues may cause cancer by interacting with biological components including RNA or DNA.

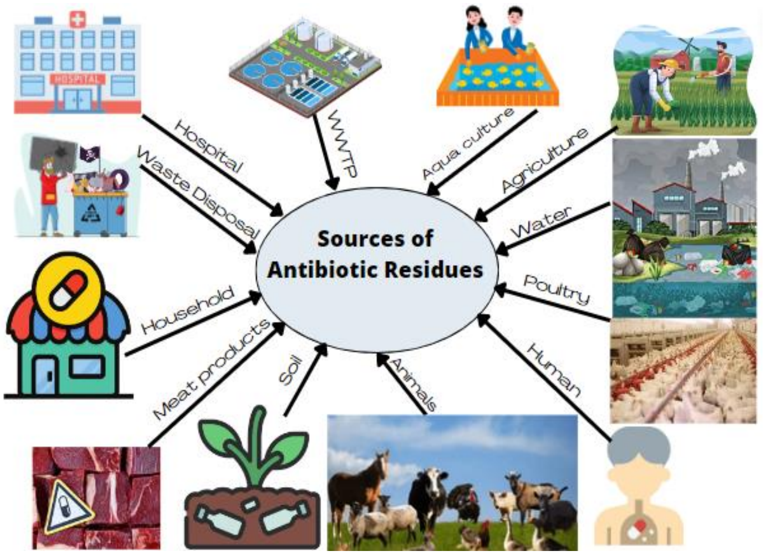

Fig. 4: Major sources of antibiotic residues

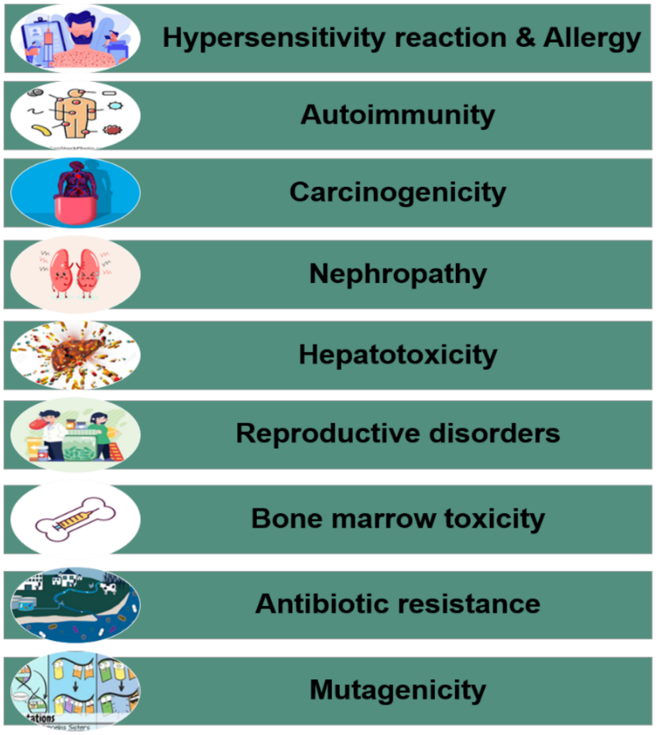

Fig. 5: Public health risk due to antibiotic residues

- **Mutagenicity:** Another negative effect of antibiotic residues is their mutagenic effect, which can provoke DNA mutation or chromosome dysfunction. Human infertility may result from these alterations.
- **Teratogenicity:** Due to the presence of antimicrobial residues for an extended time during gestation, several inborn defects may be seen in the newborn child.
- **Disruption of the usual Condition of the Intestinal:** The intestine's normal flora coexists with diverse microorganisms and fills its space to prevent illnesses caused by pathogenic microorganisms. A large number of normal intestinal flora that has non-pathogenic microorganisms may be destroyed by antimicrobial residues, by using a broad range of antibiotics. This can highlight disease-causing microbes and disturb the normal intestinal flora (Kyuchukova 2020).

Antimicrobial Residues: A Leading Cause of Antimicrobial Resistance

The antibiotic residues may cause the mutation in susceptible bacteria prolonging the survival of bacteria and promoting their multiplication as drug-resistant bacteria which carry the resistant genes.

The transferable genetic elements, involving integrons, prophages, and plasmids, will promote the spreading and raise of genomic recombination of antibiotic-resistant genes by conjugation, transduction, and transformation, together known as horizontal gene transfer (Ben et al. 2019).

The main cause impelling the rise of antimicrobial resistance include duration of exposure to antimicrobials, drug concentration, host immune status, organism type, and antimicrobial type (Ngangom et al. 2019).

The transmission of antimicrobial resistance among the environment, animals, and humans occurred through many routes. Among them environment act as a bridge between several components of human and animals to water, sewage, soil and air. The ecosystem serves as a reservoir to blend mobile genetic components that spread and interact with other parts of the environment or animal and human hosts. The spread of antimicrobials arises through numerous ways such as the manufacturing industry, hospital, and municipal waste, landfill leachates of antibiotic discharge, and surface runoff from agricultural fields comprising livestock manure and animal husbandry. The antimicrobial compounds have a half-life ranging from minutes, and hours to several days but antimicrobial residues are determined as the permanent part of the environment (Ahmad et al. 2021).

Impact of Consuming Antibiotic Residues Containing Food and Antibiotic Resistance

The World Health Organization promotes the idea of "one health," which encompasses both animal and human health. It highlights the broad relationship between the environment, animals and humans, all of these direct the distinctive approach to health. Thus at all of these three levels, the entire chain should consider maintaining equilibrium by using medicinal products carefully. Nowadays there is much public awareness about the effects of increased and prolonged use of antibiotics in the production of animal livestock. Antibiotic resistance genes can be developed in microorganisms, which is helpful in their survival, minimizing treatment of microbial infections, selection pressure, and increasing mortality in humans. A fundamental source of antibiotic residues is animal food which is a result of the indiscriminate use of antibiotics at the industrial level. Worldwide differences in antibiotic-resistance genes and the prevalence of animal-based antibiotic-resistant bacteria have been reported between different geographical areas. Even after the heat treatment antibiotic residues remain in animal-based food such as eggs, meat, and milk and cause antipathy and gastrointestinal diseases in consumers or lead to the appearance of superbugs because of antimicrobial resistance. Antibiotic resistance as well as the ineffectiveness of antibiotic therapy is increasing in humans. It is mentioned in the literature that the abusive and constant use of antibiotics can induce the production of antibiotic-resistant bacteria. Infections of multidrug-resistant bacteria can pose a threat to public health by increasing mortality. The researchers have mentioned that after a while, bacteria will develop resistance to compounds similar to antibiotics such as phenolic compounds (Ghimpețeanu et al. 2022).

Kumar et al. (2020) investigated the topic to comprehend more promising mechanisms for developing or spreading antibiotic-resistant genes in clinical, nutritive, agricultural, and environmental settings. Alternate dietary plans were also assessed to replace medicated feed and antibiotics with prebiotics, probiotics, antibodies, and essential oils having a prophylactic role against infections caused by microorganisms. Animal husbandry is a curtail contributor to food requirements and a significant component of the global economy. Antibiotics are not only given to animals through feed but also in drinking water for treatment purposes or to gain weight. Various preventive measures should be taken globally to eliminate the resistant bacteria such as avoiding the use of antibiotics for non-therapeutic purposes. Antibiotics should be strictly avoided when animal food is intended for the consumption of humans (Singh et al. 2014).

Avoparcin has been banned in European Union to reduce the occurrence of antibiotic resistance in humans and animals (Ghimpețeanu et al. 2022). To control antibiotic resistance transmission from farm to fork, the expansion of the World Health Organization or Food and Agriculture Organization of the United Nations, Global action plan is necessary along with one health concept. Infections caused by antibiotic-resistant bacteria kill 33,000 people every year. WHO prohibited the use of antibiotics for animals that produce food and fully restricted the prevention of disease using antibiotics without the diagnosis. Several countries are already taking measures to decrease antibiotic usage in food-producing animals. European Union has banned the use of antibiotics for growth promotion since 2006. Additionally, consumers also promote the marketing of meat from animals that are raised without using antibiotics, some food chains also supply meat with “antibiotic-free” policies. The main objective is to use antibiotics when it is necessary so that the effectiveness of antibiotics is maintained for treatment purposes (WHO 2022). FDA listed 89 antibiotics that are medically important and added to the feeds of animals. Furthermore, national programs for the inspection of antibiotic residues in different types of food products are being updated constantly (Mensah et al. 2014).

Environmental Risk

The environmental occurrence of antibiotic residue is a result of countless configurations of antibiotics normally subjected to combat microbial infections and increase the production of livestock. The antibiotics use in the treatment of animals and humans are reaching the environment settings through waste matter discharged from the body, especially feces and urine. About 40 to 90% depends upon the type of antibiotic excreted in active form, returning to the atmosphere and contaminating the water, soils, fields, plants, air, etc. (Oloso et al. 2018). The routine usage of huge volumes of antibiotics in animal farming can result in the contamination of farmed lands through the use of adulterated manure on fields as fertilizer and the wastewater used for the irrigation of crops. Further concern rises from the inappropriate disposal of antimicrobial products into sewage systems. Many wastewater treatment plants are working to treat antibiotic-contaminated water but 100% removal of antimicrobials is not possible, therefore the final effluent or sludge contains antibiotics in excess amounts. This sludge is then used in plants as a fertilizer and discharged in water and this water might end up in drinking water sources like lakes and rivers (Polianciuc et al. 2020).

Apart from livestock and poultry, human dwellings, hospitals, and aquaculture farms antibiotic contaminated wastewater are occasionally released into the environment without undergoing any treatment. In many countries, the routine monitoring of the antimicrobials and their residues in drinking water is not possible due to the high cost (Lundborg and Tamhankar 2017).

Technological Risks

The antibiotic residues pose a major technological risk to food products processed by microbial fermentation for example in fish (shrimp paste, bagoong, surströmming, gravlax, fish sauce, garum and fesikh), meat (salami, corned beef, sausage, chorizo, soudjouk, and pepperoni) and milk (cheese and fermented milk product). The interference of antibiotic residues in these raw materials can hinder the fermentation process by obstructing the starter cultures, leading to poor quality of food and manufacturing faults (Arsène et al. 2022).

Current Detection Method of Antibiotic Residues

Traditionally the detection of antibiotic residues can be divided into three groups: chromatography/mass spectrometry detection techniques, immunoassay techniques, and microbial inhibition screening techniques (Wang and Zhao 2018). The widely and earliest-used method is microbial inhibitions assays. These assays are cost-effective and cover a broad spectrum of antibiotics in one go. There are two types of tests, one tube test, and the other agar plate or microtiter well plate test. The main principle of these tests includes spore suspension of the most highly sensitive bacteria in the growth medium, accompanied by an indicator (pH or redox). The bacterial growth begins at the optimum temperature and acid production by fermentation is indicated by color change. The absence of color change indicates the presence of antimicrobial residues that prevent the growth of bacteria (Pikkemaat 2009).

The confirmatory methods with high specificity are laborious, expensive, and need an adequate or personnel laboratory. These methods are essentially chromatographic techniques (mainly liquid chromatography) coupled with ultraviolet (UV) or mass spectrometry. Furthermore, enhanced surface Raman spectroscopy, high-performance liquid chromatography (HPLC) with a spectroscopic HPLC–photodiode array detector or with spectroscopic fluorometric detection (HPLC–RF) and capillary electrophoresis (CE), CE– laser-induced fluorescence, are also presented to be efficient in detecting antimicrobial residues. Now a day's fully automatic highly specific onsite application biosensors are becoming progressively significant in identifying antibiotic residues in various foodstuffs. These biosensors can be categorized based on biological element (microbiological, enzymatic, and immunosensory), transducer (calorimetric, piezoelectric, thermal, optical, electrochemical, and impedimetric) and immobilization of biological element procedure on solid support medium (encapsulation, adsorption, entrapment, cross-linking and covalent bonding) (Arsène et al. 2022).

Control of Antimicrobial Residues

The following steps may be taken to lower the risk of antimicrobial residues on animal and public health:

- The most important and initial step is educating people about the issue through various organizations, literature, government agencies, and veterinary personnel.
- Rapid analysis of all antibiotics spectrum in one test.
- Proper maintenance and monitoring of MRL and withdrawal time.
- Promote ethnoveterinary practices.
- Cost-effective and simple field test to determine the antimicrobial residues in food-producing animals.
- Ban food containing antimicrobial residues.
- Use of resins, UV irradiation, and activated charcoal help in the inactivation of antibiotics.
- Avoidance of irrational use of antibiotics in the field.
- Periodic surveillance and monitoring of the residues in food animals and their products (Nisha 2008).
- Antibiotics used as growth promoters should be banned.
- Only prescribed antibiotics should be given to animals by the veterinarian.
- For antibiotic therapy, a narrow-spectrum antibiotic should be chosen (Okocha et al. 2018).
- Alternative treatment strategies should be adapted for example probiotics and plant base elements (Bacanlı and Başaran 2019).

Conclusion

Allergies, cancer, altered intestinal flora, bacterial resistance, and the suppression of fermentation in the dairy sector are all possible outcomes of antimicrobial residues in foods of animal origin. The potential development of antimicrobial resistance by antimicrobial residues in meat, milk, egg, and fish poses a severe hazard to public health. There may be consequences for the food supply system, which is already under stress from the ongoing epidemic of COVID-19. The scientific data needed to advise political decision-makers to interact with veterinarians and livestock producers, and promote sustainable development plans. Health officials may take notice and begin formulating strategies to reduce or eliminate this risk.

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2

Bacterial Biofilms and their Regulatory Approaches

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INTRODUCTION

Bacteria have been intensively researched in their planktonic condition from the beginning, yet in natural systems they seldom exist in an autonomous, free-swimming planktonic state. Instead, they reside in interdependent complex multicellular surface-attached communities called biofilms that are bound together by a self-secreted extracellular polymer matrix mostly made of polysaccharides, secreted proteins, and extracellular DNAs (Tremblay et al. 2013). Antoine Von Leeuwenhoek initially saw a particular species on his own teeth in the 17th century, a discovery known as a biofilm (Jamal et al. 2018), however, the term "biofilm" was coined in 1978 by a Canadian scientist named "Bill Costerton." According to his definition, a biofilm is a heterogeneous structure made up of several populations of microorganisms encased in an extracellular matrix that allows bacteria to cling to either an abiotic (such as rock, glass, or plastic) or biotic surface (skin, mucosa, or cuticle) (Berlanga and Guerrero 2016).

The myth that the majority of bacteria on Earth are capable of building biofilm is generally believed since microbes are ubiquitous and can be found everywhere and on every surface. In contrast to the matrix, which may make up more than 90% of the dry mass, microorganisms make up less than 10%. In various conditions, between 40 and 80 percent of bacteria on earth are capable of forming biofilms (Flemming and Wuertz 2019). The extracellular matrix is primarily responsible for the resilience of bacterial biofilm. It has structural and functional roles in the production of vital nutrients, extracellular enzymes, cell communication, and defense against various hazardous substances such as antimicrobials and disinfectants (Galié et al. 2018).

Bacteria develop biofilms to survive under certain environmental circumstances. Depending on their composition, biofilms may be made up of a single homogeneous species (monomicrobial biofilms) or a variety of heterogeneous/diverse species (polymicrobial biofilms), sometimes crossing several biological kingdoms. In particular, cross-kingdom polymicrobial biofilms have been shown to be more resistant to antimicrobial therapy. In general, they can be conceptualized as porous, soft viscoelastic materials similar to hydrogels (Raghupathi et al. 2018).

Reason of Biofilm Formation

Bacteria develop biofilms in response to environmental stresses such as insufficient/low nutrients, high temperatures, high pH, high salt concentrations, high pressure, ultraviolet radiation, desiccation, and antimicrobials. In contrast to their planktonic cell life, microorganisms may thrive in oligotrophic environments with greater ease, have better access to nutrients, are protected from biocides, have increased productivity and interactions with other organisms, and have more stable environments due to the development of biofilms. Under certain situations, biofilms protect them and make them more adaptable to the external environment (Galié et al. 2018).

Biofilm Formation Process

A central belief of biofilm formation is that it is a dynamic process. It depends on how the bacterium cells, substrates, and nearby environments interact (Hall-Stoodley and Stoodley 2002). Biofilm formation models depict it as a linear process, however, the events involved in its formation are complex having multiple steps and being regulated by the intracellular and intercellular signaling systems. Free-floating bacterial cells initially connect reversibly to a surface with the help of intermolecular forces and hydrophobicity, followed by an irreversible attachment that is typically assisted by bacterial adhesive structures and close proximity interactions and finally the creation of EPS (extracellular polymeric substances) that allow cells to cling firmly to a

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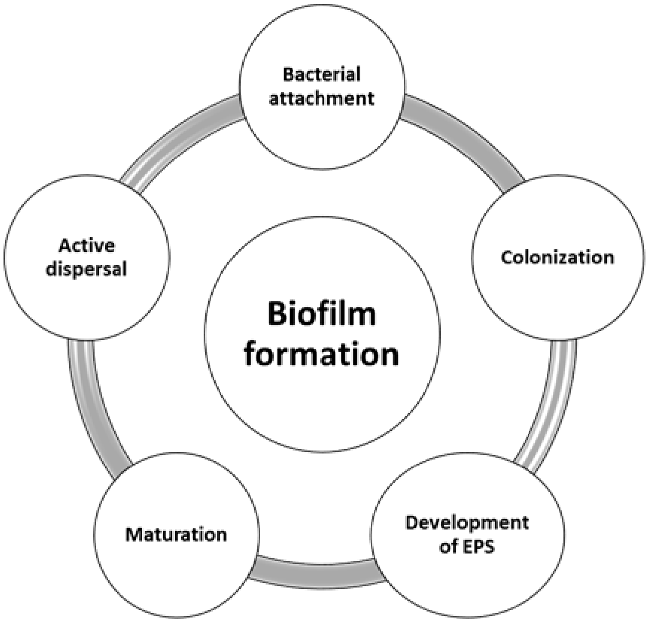

Fig. 1: Steps involved in biofilm formation

medium's surface. Eventually, they become a structured entity contained within an EPS grid. Lastly, bacteria can break free from the developed biofilm and spread in the environment to inhabit fresh niches. So in between initiation and termination of biofilm formation there are defined specific biofilm stages, but the currently available evidence suggests that these transitions are mainly governed by adaptive responses, and not by specific genetic programs (Yu et al. 2016).

Thus, there are five different stages that plays pivotal role in biofilm formation process

1. Reversible attachment
2. Irreversible attachment
3. Extra-cellular Polysaccharide (EPS) production
4. Biofilm Maturation
5. Detachment/Dispersal

Through these stages, free-living planktonic bacteria transition into "biofilm" lifestyle bacteria. Fig. 1 illustrated the steps involved in the formation of biofilm.

1- Reversible Attachment

The preliminary step of biofilm formation is reversible attachment of bacteria to a substrate. It occurs after an interaction with the abiotic or biotic substrate surface and few bacterial cells under specific environmental conditions. Bacterial cells migrated to substrate surface via chemotaxis, sedimentation, and Brownian motion (Palmer et al. 2007). Chemotaxis enables the enhanced interactions between bacteria and surface by directing the movement of bacteria towards the nutrient source via chemical signals (Vladimirov and Sourjik 2009). Once the bacteria interact with the surface, attachment of bacteria depends on the forces which are either attractive or maybe repulsive. Bacteria will adhere to surfaces if appealing forces outweigh repelling forces, and vice versa (Carniello et al. 2018). The main forces behind the preliminary attachment of bacteria and the surfaces are Van der Waals molecular interactions, electrostatic forces, and hydrophobic interactions (Dunne 2002). This attachment of bacteria is reversible and occurs when bacteria adhere to the surface of medium in a way that bacteria stay in a 2d Brownian motion. At this stage, bacteria can be detached from the surface either by their own motion or movement or by the flow of any fluid or liquid over the surface of substrate (Li and Tang 2009).

Any surface (biotic or abiotic) coming in the contact with bacteria can act as a substrate for the bacterial attachment (Tuson and Weibel 2013). Bacterial attachment is majorly affected by hydrophobicity, roughness, and charge of surface. It is observed that hydrophobic surfaces tend to have more bacterial colonies than hydrophilic surfaces. This may be due to less repulsive forces on hydrophobic surfaces than hydrophilic surfaces (Yu et al. 2016). Roughness of substrate surface also plays a major role in the bacterial attachment. The spaces and irregularities between substrate surface can promote the bacterial attachment because of more surface area to which bacteria can adhere (Zhao et al. 2014). Thirdly, surface charge can also affect the adherence of bacteria to the surface of substratum. The significant quantity of amino, carboxyl, and phosphate groups found in bacterium cells contributes to the cells' increased negative charge. That's why surfaces with positive charge promotes the adhesion of negatively charged bacterial cell (Dziubakiewicz et al. 2013).

2- Irreversible Adhesion

The second step in biofilm formation is irreversible attachment or adhesion of bacterial cells to the substrate surface. This step is accomplished with the help of short-range interactions namely;

- Covalent bonding
- Hydrophobic interactions
- Ionic bonding
- Hydrogen bonding
- Dipole-dipole forces (Bos et al. 1999)

The bacterial surface is covered with many adhesive projections that are projected away from the extracellular environment. In biofilm formation, many bacterial adhering structures take part mainly; pili, flagella, fimbriae, and adhesion initiated by non-fimbrial structures. The first physical contact of bacterial cells with the substrate surface is achieved using these adhesive structures (Berne et al. 2015).

Irreversible attachment using flagella is one of the most common ways of forming biofilm in bacteria. Flagella is a whip like filamentous thread plays a role in the locomotion of bacterial cell. Flagellar movement can be of either swarming (on solid surface) or swimming (in liquids). A wide range of bacterial species shows both type of movement for the attachment on the surface of substrate (Hintsche et al. 2017). Flagella plays a major role in overcoming the repulsive forces that can obstruct the bacterial attachment and biofilm formation and initiates the initial adhesion of cells (Terashima et al. 2008).

Another filamentous appendage used by bacteria for the attachment of cells with each other and initial adhesion is pili or fimbriae. Some bacteria can utilize a pili mediated movement known as twitching motility that can play a role in initial adhesion (Maldarelli et al. 2016). On their exterior, some bacteria have type 1 fimbriae and type 3 fimbriae. These fimbriae are very helpful for biofilm formation and adherence (Murphy et al. 2013). In addition, curli fimbriae, which are thin and flexible, aid in bacterial attachment in the onset (Carter et al. 2016).

3- EPS Production

Third step in the biofilm formation is production of EPS (extracellular polysaccharide). Bacterial cells produce and secrete extracellular polysaccharide for biofilm formation. EPS is a very important constitute of biofilm extracellular matrix. EPS regulates both adhesion of biofilms to substrate surface and cohesion of bacterial cells by using two main forces i.e., Ion bridging interaction and hydrophobic interaction. Bacteria produce EPS because of many reasons including; attachment to the surface of substrate, intra-cell recognition, formation of biofilm, structural constitute of biofilm, cell signaling, protection of cells from desiccation, trapping of nutrients, and for exchanging genetic information to other bacterial cells (Fahs et al. 2014). A secondary messenger known as c-di GMP functions as a stimulant to control the transition of bacterial cells from reversible attachment to permanent adherence. The production and release of EPS and other cell structures enable this change (Toyofuku et al. 2016).

EPS produced by bacterial cell to form biofilm is made up of polysaccharides, DNA, lipid, proteins, and other compounds. The most important and prominent constitute of bacterial EPS is polysaccharide which plays a major role in the maturation of bacterial biofilm and their growth (Bacosa et al. 2018).

In Gram-negative bacteria, polysaccharide present in the EPS is either neutral or polyanionic. Uronic acid present in the polysaccharide is considered to be the reason of this anionic nature. This nature of EPS clears the way for attachment of divalent cation molecules like calcium and magnesium which facilitates in the formation of biofilm. On the other hand, Gram-positive bacteria produce EPS which is cationic in nature (M. Donlan 2002) . The extracellular matrix (EPS) of gram-positive bacteria is made up of many different types of molecules, including proteins, DNA, enzymes, and even structures like pili and fimbriae. DNA present in the EPS acts as intracellular connector (Flemming et al. 2016).

4- Biofilm Maturation

Changes in gene expression are triggered during microcolony formation and EPS accumulation, and the products of these genes are used to produce EPS, which acts as biological "glue" between implanted bacterial cells (Karimi et al. 2015). The creation of the matrix is succeeded by the production of water-filled channels that function as circulatory systems, transporting nutrients to the cell communities and eliminating waste. According to a structural study, microcolonies have a pyramid/mushroom-shaped multicellular structure (Garnett and Matthews 2013). During the maturation process, mobility within microcolonies is limited because surface structure development in bacteria is inhibited. Additionally, differences between the gene expression patterns of sessile and planktonic cells are striking (Hall-Stoodley and Stoodley 2002). Furthermore, QS allows bacteria of the same or different species to communicate by secreting and detecting AIs. Bacteria employ these signaling molecules to detect one another presence and to control gene expression in response to variations in population density (Wei and Ma 2013).

5- Dispersal/Detachment

Detachment of bacterial cell from biofilm is also called dispersal of biofilm. It is the final and terminal phase of biofilm formation. Bacteria detach from the already developed biofilm to continue a new cycle of biofilm (Singh et al. 2017). Bacterial cell dispersal from the adhered biofilm surface is due to environmental factors under which bacteria form new microcolonies on another substrate surface. This final step is rather complex because it is influenced by signal transduction pathways and environmental signals and factors (Díaz-Salazar et al. 2017).

The process of dispersal differs among different bacterial species but in general the whole process of detachment and dispersal can be classified into three steps i.e., cell detachment, movement of cells towards a new substrate and bacterial cell adhesion to new substrate Detachment of bacterial cells can be either active (seeding) or passive (erosion or sloughing). Seeding action is the detachment of bacterial cells from biofilm under the signals from environmental factors like nutrient deficiency, degradation from enzymes, antimicrobial actions. The biofilm's core is quickly evacuated by bacterial or planktonic cells during the seeding dissemination process, leaving a hollow interior. Passive detachment of cells is done under the action of external force also known as shear forces. The rapid dispersal of a large portion of bacterial cells from biofilms is known as sloughing, whereas the sudden dispersal of a tiny portion of bacterial cells from biofilms is known as erosion (Lee and Yoon 2017).

During the detachment process the genes responsible for EPS production, adhesion, and pili production are downgraded while genes responsible for cell motility and movement such

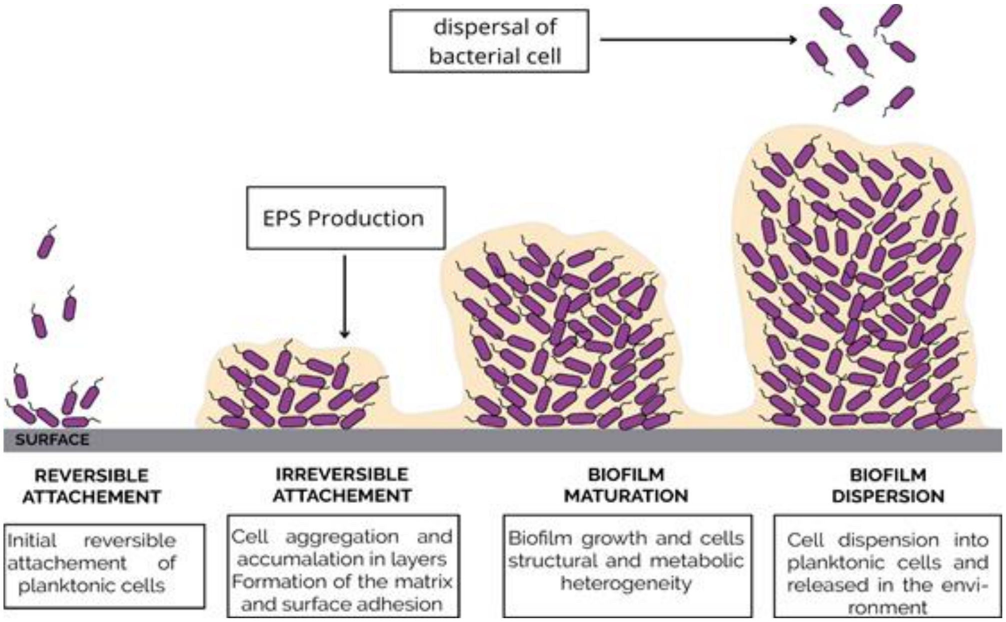
dispersal of bacterial cell
EPS Production
SURFACE
REVERSIBLE ATTACHEMENT
IRREVERSIBLE ATTACHEMENT
BIOFILM MATURATION
BIOFILM DISPERSION
Initial reversible attachement of planktonic cells
Cell aggregation and accumalation in layers Formation of the matrix and surface adhesion
Biofilm growth and cells structural and metabolic heterogeneity
Cell dispension into planktonic cells and released in the envi-ronment

Fig. 2: Process of biofilm formation

as production of flagella and EPS degradation are upgraded. Environmental factors like temperature, pH change, nutrient deficiency and oxygen scarcity plays a major role in the dispersal of bacterial cells from biofilms. Environmental factors like limited supply of oxygen contributes in the degradation of c-di-GMP which facilitates in the dispersal of biofilm (Raghupathi et al. 2018). Additionally, a physiochemical process also known as autolysis assists in the biofilm dispersal (Lee and Yoon 2017). Fig. 2 highlights the complete process of biofilm formation.

Impacts of Bacterial Biofilm

There are many harmful effects of bacterial biofilm. Bacteria can form biofilm on any surface including biotic or abiotic surfaces (Hall-Stoodley and Stoodley 2002) . There are too many impacts of bacterial biofilms like nosocomial illness, food spoilage, dental carries, ship hull fouling issues, spoilage of sea foods as well as industrial pipe-fouling (Khatoon et al. 2018).

Public Health Hazards

In hospital environment, bacteria can form biofilm on every surface including medical equipment (prosthetic heart valves, machines, pacemakers, lenses, and catheters), dead tissues and part of bones, and on living tissues (teeth and lungs surface) (Alav et al. 2018).

In health care environment both Gram-negative and Gram-positive bacteria are found attached with the surface of medical equipment and form biofilm, but the most prominent bacteria reported in the hospital environment forming biofilm are; *Staphylococcus (S.) aureus*, *S. epidermidis*, and *Pseudomonas (P.) aeruginosa*. Out of these bacteria, most of the infections caused by the staphylococcal species (Hall-Stoodley and Stoodley 2002). *P. aeruginosa* is mostly found in the water system of health care hospital (Loveday et al. 2014). Moreover, biofilm forming bacteria are main contributors of life-threatening diseases in the hospital environment like; Osteomyelitis, chronic wounds, infective endocarditis (IE), and cystic fibrosis (Masters et al. 2019). It is reported that biofilm producing bacteria accounts for about 65% of all the bacterial infection in hospital environment because they show high resistance against antibiotics and host defense system (Ciofu and Tolker-Nielsen 2019).

Impact on Food Industry

The existence of biofilm and bacteria in food industry and food processing units can have very consequential risks in food industry and for food (Galié et al. 2018). Food industry is highly affected by the biofilm forming bacteria. Biofilm producing bacteria can be attached to any surface in the food industry including food surface. Approximately 60% of all the foodborne infections arises due to the bacteria producing biofilms (Han et al. 2017). In food industry, biofilm producing bacteria mainly come from the surrounding air, contaminated equipment, and spoiled food surfaces (Coughlan et al. 2016). The biofilms formed on food surface results in the spoilage of food which is a very serious concern for consumers and can have economic and financial consequences. The most common biofilm forming agent present in the food industry and on the surface of food is *Listeria monocytogenes* which is found everywhere and can cause serious complications in the pregnant women and other infections in the immunocompromised patients. After that a major spoiler of food in food industry *Salmonella* spp. can cause foodborne diseases and leads to Reiter's syndrome or in severe cases lead to death (Galié et al. 2018).

Many technical problems also occur due to the presence of biofilm in the food industry like prevention of heat flow across the surface of equipment, increase in the resistance of fluid, increase in the rate of corrosion of surfaces, and loss of food production (Meesilp and Mesil 2019).

Benefits of Bacterial Biofilm

There are many harmful effects of biofilm in the environment, but it has some benefits too. The benefits of biofilms are primarily seen in the agricultural, bioremediation, wastewater treatment, corrosion protection, and other sectors (Singh et al. 2019).

1- Bioremediation

In the process of bioremediation, living organisms, mostly bacteria, interact with harmful and toxic substances in the environment to change them into less dangerous forms (Van Dillewijn et al. 2009). In terms of cost and safety, it is a better solution than traditional cleanup including various chemicals and physical removal of contaminants (Mangwani et al. 2015). Furthermore, biofilm-mediated remediation approaches are more efficient in changing hazardous wastes due to greater pollutant bioavailability to degrading organisms and enhanced adaptability of degrading bacteria to diverse toxic chemicals (Dos Santos et al. 2018). The process is generally a step of bacterial metabolism and depends on enzymatic assault by bacteria to transform environmental contaminants into harmless chemicals (Rodríguez-Martínez et al. 2006). Microbial bioremediation can occur at the site of contamination (in situ) or elsewhere (ex situ) (Azubuike et al. 2016). It may be accomplished by incorporating limited nutrients and electrons (bio stimulation) or by introducing microorganisms into contaminated areas (bioaugmentation) to boost the transformation process (Rodríguez-Martínez et al. 2006). Microorganisms residing in biofilms have better tolerance to toxins, a higher likelihood of survival and adaptability and stronger capacities to degrade various pollutants through catabolic pathways than their planktonic counterparts. Additionally, biofilms provide an essential habitat for bacterial chemotaxis, adherence, metabolite dispersion, and intercellular gene transfer (Dos Santos et al. 2018).

Numerous aerobic and anaerobic bacteria may thrive in biofilms during remediation processes, and these bacteria frequently use the decomposition of pollutants as a source of energy. Bacteria may employ oxygen as the ultimate electron acceptor during aerobic degradation to convert harmful pollutants into benign products, mostly carbon dioxide and water. When pollutants are transformed into less harmful or innocuous byproducts in anaerobic settings, electron acceptors like nitrate and sulphate, which function like oxygen, may be necessary for the byproduct's survival (Azubuike et al. 2016). Pesticides, dyes, heavy metals, explosives, medicinal products, and chronic organic pollutants, includes compounds such as PAHs, PCBs, and PCEs (polychlorinated biphenyls and polychlorinated ethenes) are all potential candidates for bacterial biofilm-mediated cleaning (Edwards and Kjellerup 2013).

2- Wastewater Treatment

Water contamination is a significant concern worldwide as a result of industrialization, population expansion, and urbanization (Daud et al. 2017). There are many different types of organic and inorganic pollutants found in wastewater, which are brought on by industrial sewage, domestic and commercial sewage, storm water, and other sources. The protection of aquatic environments depend on wastewater purification (Naidoo and Olaniran 2013). Electrochemical treatment, membrane filtering devices, and coagulation/flocculation are all physicochemical wastewater treatment techniques (Favero et al. 2020). These processes are effective, but they struggle to remove organic matter because filtering and sanitation are the two main elements of most water treatment systems (Hlihor et al. 2017). Biofilm-based wastewater treatment systems have made use of microbial communities to degrade and neutralize organic and inorganic contaminants. To stop marine eutrophication, which results in anoxia, extra nutrients in effluent must also be eliminated. Typically, nitrogen and phosphate are the main nutrients in effluent. As a consequence, denitrifying or phosphorous-neutralizing bacteria are frequently used in wastewater purification (Yamashita and Yamamoto-Ikemoto 2014).

The biologically active carbon (BAC) method, which is one of the biotechnologies used to purify water, physically removes pathogenic bacteria, organic matter, and inorganic pollutants from water by using granular activated carbon (GAC) as a water filtering medium (Shirey et al. 2012). To degrade organic carbon and other contaminants in the influent water, bacteria colonize and form biofilms on the rough, porous surfaces of the GAC after the media granules have been depleted. Some of the biofilm reactors being developed at the moment for the remediation of wastewater include revolving contactors, fluidized beds, and membrane reactors (Simpson 2008).

Regulatory Approaches of Biofilms

Unlike planktonic bacteria, biofilms are not effectively eliminated by normal cleansing, washing, and disinfection methods, however, the creation of biofilm may also be helpful (Somers and Lee Wong 2004). As a result, a variety of elements have been investigated to encourage the creation of beneficial biofilms. Bacterial biofilm matrix, quorum sensing interference, and regulation of bacterial attachment

Table 1: Regulating strategies of bacterial biofilm and their mechanism (Chung and Toh 2014)

Strategy	Mechanism
1. Prevention and promotion of bacterial attachment	
• Use of polythene glycol (antifouling surface)	Repel bacterial coating
• Use of silver (antimicrobial surface)	Bacterial killing while in contact with surface
• Use of aryl rhodanines and other small molecules	Non-adhesive property
• Use of curlicides and pilicides	Non-adhesive property
Modifying surface of substratum	
• Oxygen plasma on carbon materials	Promotes bacterial adhesion and bacterial biofilms
• Nitrogen plasma with carbon anode	Enhance bacterial biofilm production
• Use of polythene membrane	Enhance nitrifying bacteria forming biofilm
2. Promotion and Control of bacterial signal transduction	
• Quorum quenchers	Degradation of signal molecules using enzymes
• Quorum sensing inhibition	Inhibit synthesis of signals
• Natural agents (Furanone, ajoene. Etc.)	Degrade biofilm formation
• AIs and QS genes	Encourage the formation of beneficial biofilm
• Increased expression of QS gene	Enhance EPS formation
3. Dispersal of biofilm matrix	
• Targeting matrix enzyme	Degradation of EPS
• Use of Bacteriophages	Degradation of EPS
• C_2DA (Cis-2 decenoic acid)	Enhance biofilm dispersal

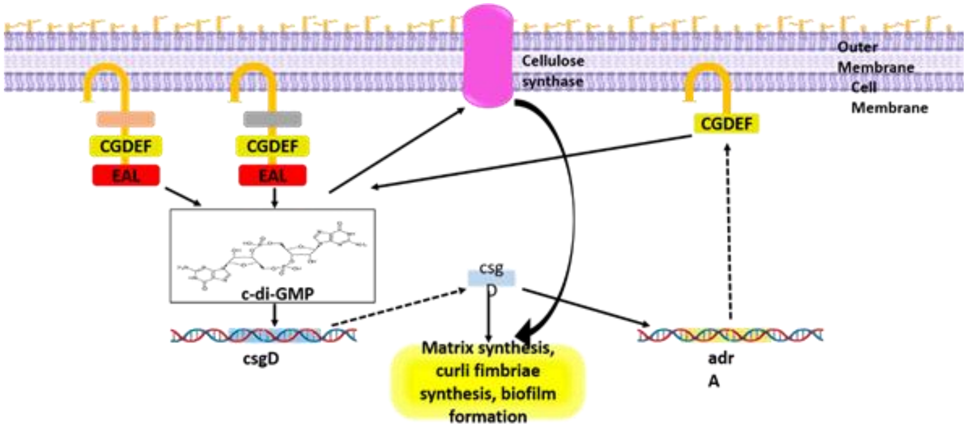

Fig. 3: Regulation pathway of biofilm formation by DGCSs.

are all closely linked (Chung and Toh 2014). Various strategies for avoiding, regulating, or encouraging bacterial biofilm development are enlisted in Table 1. Regulation pathway of biofilm formation by DGCSs is shown in Fig. 3. Various diguanylatecyclases act both upstream and downstream in the signaling cascade that regulates biofilm formation. Broken lines connect the genes to their gene products.

Prevention and Control of Bacterial Attachment

The best way to stop the production of biofilms in their early stages is to inhibit cell attachment. As a result, redesigning the surface or covering the surface with compounds that inhibit bacterial adherence may limit the creation of bacterial biofilm (Chung and Toh 2014). There are two kinds of antibiofilm surfaces i.e., antifouling surfaces and antimicrobial surfaces. In contrast to the latter, the former prevents pathogens from sticking to surfaces and prevents the growth of microbes there (Xu et al. 2005). Antifoulants in the form of nanoparticles of titanium oxide, graphite, arsenic, mercury oxide, silver, copper oxide, and zinc oxide have been manufactured and employed successfully in coating agents and coatings (Kuang et al. 2018). Recently, the marine and healthcare industries have started to use polyethylene glycol (PEG) on a large scale as an antifouling coating. PEG-coated surfaces have been discovered to inhibit bacterium adhesion due to their hydrophilic surface characteristics. Numerous bacterial species, such as *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *E. coli*, may be rejected by PEG coverings (Zhang et al. 2017). Antibacterial surfaces are created for indwelling medical devices (like catheters and intubation tubes) that may be infiltrated by bacteria that form biofilms so that pathogens including *S. aureus*, *E. coli*, and *P. aeruginosa* can be combated by releasing antibiotics, bacteriocins, metal ions, plant extracts, or nanoparticles (Chung and Toh 2014). Silver coating may be used to prevent biofilm growth on medical device surfaces. Quaternary ammonium compounds (QACs) are also utilized in contact killing surfaces functioning as antimicrobial agents. Unlike silver ions, which release antibiotics slowly over time, the QACs coatings provide a durable contact-based antibacterial action (Francolini et al. 2017). One drawback of contact killing surfaces is that some bacteria can get resistant to them (Achinas et al. 2019). Aryl rhodanines are tiny substances that prevent bacteria from adhering to surfaces and may stop Gram positive diseases from forming biofilms in their early phases (Chung and Toh 2014). Furthermore, tiny synthetic chemicals known as pillicides and curlicides may disrupt bacterial adherence by blocking the formation of bacterial pili and fimbriae. Honey and tea are natural items that may help hinder bacterial adhesion (Kuang et al. 2018).

Promotion of Bacterial Attachment

However, surface modification of materials, including both physical and chemical-based alteration, or electrochemical oxidation treatment, may encourage the adhesion of beneficial microorganisms (Kang et al. 2014). Changes and management of surface properties can promote bacterial adherence and the production of useful biofilms for BESs and the yeast fermentation sector. It has been shown that nitrogen or oxygen ions increase the hydrophilicity and surface energy of carbon-based materials, such as graphite electrodes, which in turn encourages bacterial adherence, the development of biofilms, and the production of electricity in BESs. Additionally, the application of nitrogen plasma to the carbon anode may modify its surface roughness and hydrophobicity to encourage the growth of biofilms and increase the generation of power in MFCs. Additionally, UV/O3-treated carbon felt electrodes may promote *Shewanella oneidensis* MR-1 adhesion and biofilm growth, resulting in an increase in electron transfer rate and a rise in the amount of current produced in MFCs (Berlowska et al. 2013). Ammonium is converted to nitrate as a crucial step in the treatment of wastewater (nitrification). However, the nitrifying organisms have relatively slow growth rates and do not produce robust biofilms. As a result, efforts must be taken to keep these nitrifiers in reactor systems. It has been claimed that the connection between adhering bacteria and the surface of the material determines biofilm strength and shear resistance (Lackner et al. 2009). To encourage nitrifying bacteria attachment to a membrane surface, a variety of techniques have been employed. It was found that nitrifying biofilms quickly formed on the PE membrane that had its surface altered with positively charged graft polymer chains. This membrane had a higher level of bacterial adhesion than the original, unmodified membrane. They used a combination of plasma polymerization and wet chemistry to add PEG strands with two different functional groups (-PEG-NH2 and -PEG-CH3) to the membrane surfaces. They discovered that adding methoxy-PEG-amine (-PEG-NH2) to both a smooth PE surface and a rough PP surface greatly accelerated the development of biofilms. Methoxy – PEG - amino amine's group draws nitrifiers like *Nitrosomonas europea* and *Nitrobacter winogradskyi*, facilitating the growth of biofilms (Lackner et al. 2009). Fig. 4 illustrated the schematic presentation of physiological functions of c-di-GMP.

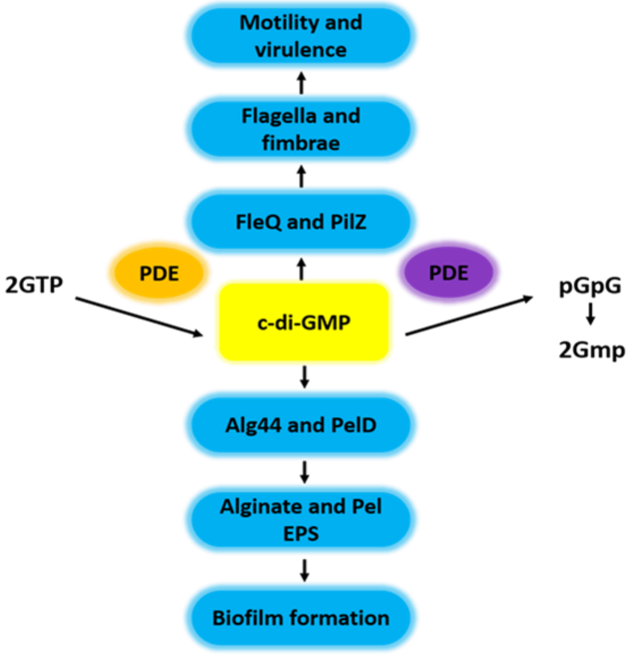

Fig. 4: Schematic presentation of Physiological functions of c-di-GMP

High levels of c-di-GMP however favours sessility and stimulate synthesis of various EPS, such as Pel (mediated by PelD) and alginate (mediated by Alg44).

Control of Bacterial Signal Transduction (Quorum Sensing)

Bacterial QS is dependent on a variety of processes, including signal generation, diffusion, receptors, and recognition, as well as gene expression and signaling reaction. Because of this, quorum quenchers (QQs) or quorum sensing inhibitors (QSIs) that thwart these procedures may be able to lower bacterial QS and eventually stop the development of biofilms (Rémy et al. 2018). Healthcare settings and medicine, as well as commercial membrane bioreactors, agricultural production, and aquaculture, all depend on the quorum-quenching method, which uses quorum quenching enzymes to inactivate quorum sensing signals. Different kinds of animals have been found to contain quorum-suppressing enzymes, including lactonase, acylase, oxidoreductase, and paraoxonase. Aryl homoserine lactone molecules are rendered inactive by QQs, which is their well-known mode of action. Another method is the QSI N-octanoyl-L-HSL (C8-HSL), which blocks the production of signal molecules (like AHLs), by inhibiting the enzymatic activity of the Lux operon protein. Among the natural QSIs that are known to stop bacterial colonies are furanone, ajoene, naringin, musaceae and curcumin (Fong et al. 2018). Additionally, using honey as a natural preservative can stop the growth of biofilms by blocking the activity of genes like AI-2 and LsrA that are involved in bacterial transmission when used in significant quantities. Additionally, more second mediator c-di-GMP encourages the development of bacterial biofilms. To stop the growth of biofilms, it may therefore be advantageous to inhibit the c-di-GMP pathway (Sharahi et al. 2019).

Promotion of Bacterial Signal Transduction

However, genetically modifying AIs can promote the growth of advantageous biofilms, which are essential to produce wastewater treatment, bioremediation, and power generation. For instance, by incorporating a specific type of AIs, the start-up time for a dual chamber MFC was reduced from 10 days to 4 days (Chen et al. 2017). The extreme halophile Halanaerobium boosts its biofilm mass on the MFC anode in response to an AI (100 mM quinolone), which improves power production and the effectiveness of high salinity wastewater treatment. Additionally, QS bacteria have the ability to degrade a wide range of pollutants. The QS genes *las*I and *rhl*I, which assist in accelerating the breakdown of polycyclic aromatic hydrocarbons, can be upregulated in order to increase the development of maritime *P. aeruginosa* N6P6 biofilms and EPS production (Mangwani et al. 2015).

Disruption of Bacterial Biofilm Matrix

It's crucial to eliminate the EPS' structural elements in order to disperse bacterial biofilms (Wei and Ma 2013). Therefore, degradation of the EPS matrix may be a useful technique to prevent the formation of bacterial biofilms. The enzymes which play a major role in EPS matrix-degradation like hydrolases, glycosides, restriction endonucleases, dispersin B, and proteases can promote the spread of pre-existing biofilm populations while inhibiting the formation of bacterial biofilms. The bacteria are released as planktonic cells after the biofilm matrix is enzymatically broken down, and these cells are quickly destroyed by phages, immune systems, antibacterial agents, and other disinfectants (Parrino et al. 2019). Both diffusion and phage-derived enzymes can help phages enter the EPS matrix. In terms of removing biofilms, an engineered lytic phage with a biofilm-degrading enzyme worked better than a non-enzymatic equivalent (Simmons et al. 2018). The capacity of phage SAP-26 to penetrate biofilm matrix when coupled with the antibiotic rifampicin led to the disruption of biofilm structures. In addition to successfully causing biofilm dispersal, C2DA may also prevent the beginning of biofilm formation (Chung and Toh 2014).

Conclusion

The formation of bacterial biofilm is very organized and highly regulated process which is related to ubiquitous nature of bacteria in natural and manmade environment. The ability of bacteria to colonize on surfaces and form biofilms are considered serious issues and is related to very disastrous consequences in many branches related to food, water, pharmacy, and healthcare. Various techniques and approaches concerning the interferences against bacterial attachment, QS and destruction of biofilm matrix have been developed to get rid of harmful biofilms. However, bacterial biofilms disrupt the bio-environments beyond risk. There are numerous beneficial applications of bacterial biofilms. Biofilm-associated bacteria play pivotal roles in the transformation of health hazardous pollutants to harmless substances, the protection of plants against phytopathogens, the plant growth promoters, as well as the removal of excess nutrients from wastewater.

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3

Salmonellosis: Food-borne Plague

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INTRODUCTION

Food-borne plague or food-borne illness also termed as food poisoning is a result of the intake of food or drink contaminated with microbiological agents like bacteria, viruses, parasites, and toxins. These pathogens act as a source of vulnerable illness causing a substantial loss of time and the treatment cost is also very high. According to the World Health Organization, a huge amount of people dies every year due to diarrheal infections which occur because of contaminated food intake (Borrás S et al. 2007). *Salmonella* as a foodborne plague has prime importance in causing illness in human food (Pires et al. 2011; Shah et al. 2011). Not only live animals, but also products of animal origin i.e., meats, eggs, milk, cheese, ready-to-eat products, and vegetables are the foremost carriers of *Salmonella* (Cellucci et al. 2010). In the case of animals, *Salmonella* causes asymptomatic infections while in humans salmonellosis results in gastroenteritis and systemic diseases (Su and Chiu 2007).

Etiology

Salmonella is a Gram-negative, predominantly motile, non-spore-forming, and facultative anaerobic bacilli. Many species belonging to the genus *Salmonella*, the family Enterobacteriaceae. Genus *Salmonella* is broadly divided into major species named enterica and bongori which are further divided into subspecies. *Salmonella (S.) enterica* has six subspecies (McQuiston et al. 2008). This well-known genus of *Salmonella* is also subdivided due to the presence of special surface molecules. The surface molecules with etiological importance are O-antigen represented as O-Ag available in lipopolysaccharide and the second one is H-antigen (H-Ag) found in the flagellar complex, flagellin protein. If we focus only on the serotypes of *Salmonella*, there are twenty-five hundred different serotypes of this genus. All these serotypes of *Salmonella* can produce infections in humans, resulting in either morbidity or mortality in a large population (Grimont and Weill 2007). Table 1 shows different serotypes of *Salmonella* with respect to their host.

Transmission

Salmonella is a disaster if it is present in a population of humans or animals as it can be transmitted through both horizontal and vertical routes. In the case of vertical transmission, the *Salmonella* serotypes move from the parent to the infant (Sanabria et al. 2017). On the other hand, horizontal transmission is done by aerogens and is also transmitted through feco-oral route. Fomites, polluted drinking water, contaminated food, and cages that are not properly cleaned are the pronounced source of transmission (Yanestria et al. 2019).

As a major cause of foodborne infection, *Salmonella* is transmitted in humans through an extensive range of food products including beef, poultry, and eggs as shown in Fig. 1 (Bell et al. 2016; Ximenes et al. 2019). The pathogen transmits not only through food products, but also live birds in humans as most people are unaware of this fact about the transmission of *Salmonella* (Behravesh et al. 2014). It resides in poultry birds and is then transferred to other animals and humans resulting in a high rate of mortality (Suardana et al. 2014).

Salmonella is not only transferred through food products such as meat and eggs but it is also transmitted through contaminated water and contact with households. *Salmonella* causes severe abdominal pain, high temperature, vomiting, and diarrhea in the host (Ximenes et al. 2019).

Pathogenesis

The distal ileum and proximal colon become colonized with *Salmonella* after consuming tainted food or drink (Hocking 2003; Lönnermark et al. 2015). Salmonellosis has an infective dosage of 10^5 to 10^6 cells which is sufficient to cause infection in the small intestinal mucosa (Xu et al. 2010). The target cells are enterocytes and it reaches them through the process of chemotaxis using its flagella. After reaching the target cell, it attaches to the human enterocytes using type l fimbriae. There are two types of fimbriae named

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Table 1: Different serotypes of *Salmonella* and their specific hosts (Baumler et al. 1998).

No.	Subgroups of *S. enterica*	Specific host	Infection
1.	*S. typhi*	Humans	Typhoid fever
2.	*S. paratyphi*	Humans	Bacteremia & fever
3.	*S. typhimurium*	Humans, Bovines, Equine, Ovines, Chicken, Mice	Fever, diarrhea.
4.	*S. enteritidis*	Human, Chicken, Mice	Septicemia, fever, gastroenteritis
5.	*S. Dublin*	Bovines, Swin, Ovines	Septicemia, fever, gastroenteritis, abortion
6.	*S. derby*	Birds, Swine	Diarrhea, Bacteraemia
7.	*S. gallinerum*	Chicken	Septicemia, gastroenteritis
8.	*S. abortosovis*	Ovines	Septicemia, abortion
9.	*S. abortusequi*	Equine	Abortion
10.	*S. choleraesuis*	Swine	Fever, Bacteraemia

long polar fimbriae (Lpf) and thin aggregative fimbriae (Tafi). In the case of *S. typhi*, the attachment with the host cells is done by using type IV pili (Wagner and Hensel 2011). After the attachment process, it gets entry into the host cell through the process of phagocytosis by using its pathogenic chemicals i.e., type III secretion systems (T3SSs). This collection of pathogenic chemicals of *Salmonella* is called *Salmonella* pathogenicity islands (SPIs) (Wagner and Hensel 2011). After this, the cells of *Salmonella* are collected by macrophages, dendritic cells, and polymorphonuclear cells as they are present in the interstitial space of lamina propria after the process of exocytosis. Then the *Salmonella* cell is captured into the host efferent lymph in the mesenteric lymph nodes (Fig. 2) to stop their entry into the bloodstream (Velge et al. 2012). When *Salmonella* gets attached to the epithelial cells of lamina propria, it produces inflammatory reactions and releases pro-inflammatory cytokines. Acute inflammatory reactions brought on by pro-inflammatory cytokines resulted in diarrhea, ulceration, and the death of mucosal cells. Mesenteric lymph nodes, liver, and spleen play vital roles in the ongoing infection of Salmonellosis (Baron et al. 1996).

It has been examined that almost all subspecies of *Salmonella* produce enterotoxin and cytotoxin. The composition of these toxins resembled that of the cholera toxins. These toxins are heat labile or heat stable and are related to diarrhea (Song et al. 2013). The production of enterotoxins results in the buildup of fluid in the ligated murine ileal loop. The damage caused by cytotoxins includes inflammatory diarrhea, damage to the intestinal mucosa, and hinder protein synthesis (Hocking 2003). The toxins produced by *S. typhi* cause typhoid fever and chronic infections (Chong et al. 2017). Another investigation shows that the virulence of both the mutant *Salmonella* with toxin phenotype and wild-type *Salmonella* with toxin phenotype is almost similar (Nakano et al. 2012).

The pathogenesis of *Salmonella* infections involves the O antigen lipopolysaccharide (LPS). All components of LPS play a crucial role in *Salmonella*'s pathogenesis, although O side chains' length, structure, composition and surface roughness can have an impact on the pathogen's virulence. The pathogenicity is reduced when a whole length of chain is not produced. The resistance of *Salmonella* strains to the lytic action of the complement cascade depends on the length of the chain. The strains with smooth surfaces face a little bit of resistance while on the other hand strains with a rough surface experience more resistance. This is due to the steric barrier of *Salmonella* to the complement cascade's ability to bind (Hocking 2003).

The pathogenicity of *Salmonella* is enhanced by the virulence plasmids and virulence genes. The virulence plasmids of *S. typhimurium*, *S. Dublin*, and *S. enteritidis* cause systemic infection to the liver, spleen, and mesenteric lymph nodes. The virulence plasmids can be isolated from human or animal organs and blood (Hocking 2003).

The virulence factors of *Salmonella* are enlisted as flagella and flagellin, superoxide dismutase, and ion acquisition systems. The invasiveness of flagella increases as the motility increases and the inflammatory response has been shown by the flagellin. Superoxide dismutase of *Salmonella* affects host cells by inactivating the bactericidal reactive oxygen species. Another feature of the virulence of *Salmonella* is that it creates ion acquisition systems for the transfer of iron, magnesium, zinc, and potassium with low concentrations in the environment. Enterobactin and salmochelin are two siderophores that *Salmonella* generates. To access the host's scarce iron, these siderophores are essential. *Salmonella* also acquires a little amount of magnesium through the CorA, MgtA, and MgtB systems. For the uptake of zinc and potassium, ZnuABC and Trk systems are employed. Each of these ions is essential for *Salmonella*'s survival and pathogenicity (Ibarra et al. 2009).

Treatment

In addition to lowering the death rate linked to bacterial infections, antibiotics are essential in the treatment of infectious disorders and have significantly enhanced quality of life. Antibiotics are selective against invasive germs: which guarantees maximum eradication of the target microorganisms while causing the least possible harm to patients (Nami et al. 2015).

Typically, antibiotics are not needed to treat NTS infections. Antibiotic therapy is used against the complications like meningitis and septicemia and the frequently used antibiotics against these are ciprofloxacin, ceftriaxone, and ampicillin (Medalla et al. 2016). The antibiotics used against the life-threatening side effects of *Salmonella* specie are cefixime, chloramphenicol, amoxicillin, trimethoprim/sulfamethoxazole (TMP-SMX), azithromycin,

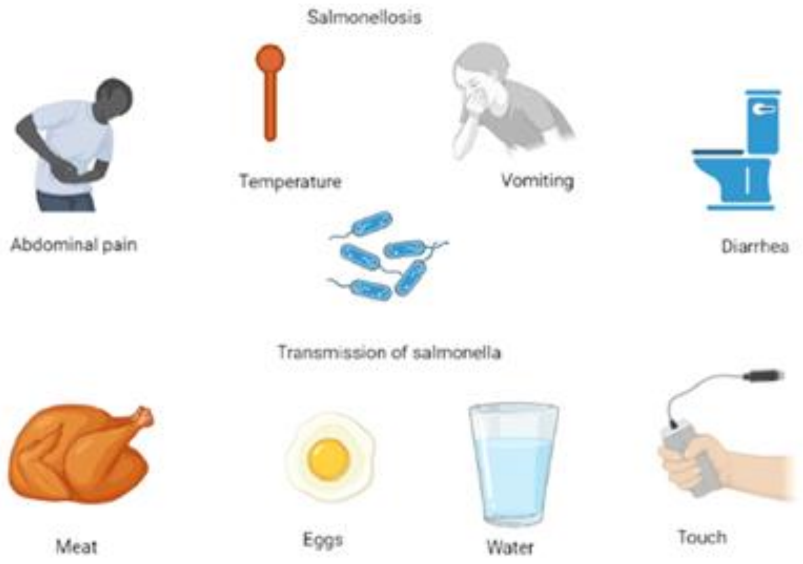

Fig. 1: Different ways for transmission of Salmonellosis.

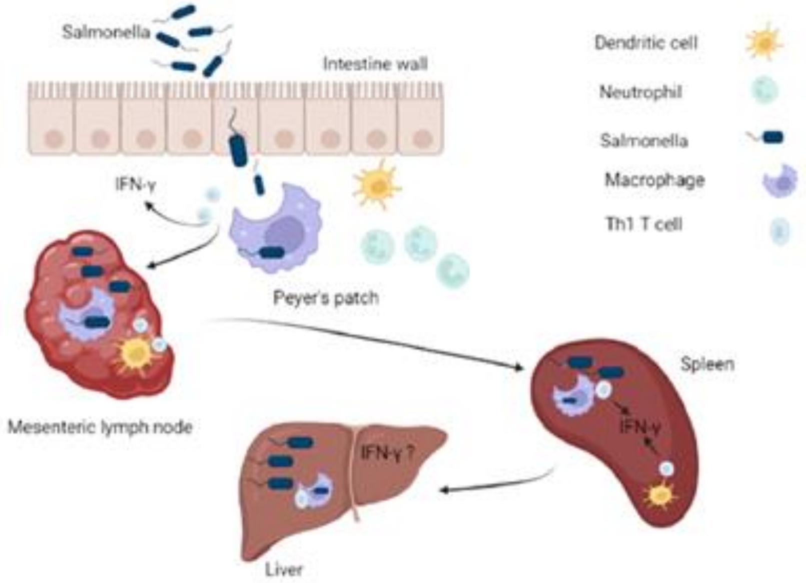

Fig. 2: Role of different organs in *Salmonella* infection.

aztreonam and cefotaxime or ceftriaxone. These antibiotics are specially used against the *S. typhi* and *S. paratyphi* to save the life of animals. Steroidal therapy is also preferred in conditions like coma, stupor, obtundation, shock, and delirium. The most common steroids used in these complications are dexamethasone and corticosteroids (Kumar and Kumar 2017).

Innovations

The use of antibiotics to treat salmonellosis produces excellent results but to some extent, the excessive use of antibiotics in patients can produce antibiotic resistance which is a global issue these days. So, the researchers develop and create different techniques and protocols to treat salmonellosis i.e., the use of yeast as a therapeutic agent against *Salmonella*. For the confirmatory diagnosis, different biomarkers are now available in the market for instance oligonucleotides.

1. Advancements in Diagnostics Techniques

The microbiological culture techniques for the confirmatory detection of *Salmonella* are generally performed but these techniques stick with low sensitivity, intensive labor requirements, time-consuming, not cost-effective, lack trained professionals and the results require weeks to confirm presence and absence of *Salmonella*. The time-efficient methods of diagnosis have prime importance as it is necessary for the treatment of clinical complications and to confirm the origin of that pathogen (Odumeru and León-Velarde 2012; Zadernowska and Chajecka 2012; Almeida et al. 2013; Ahmed et al. 2014).

2. Oligonucleotides as a Biomarker for the Detection of Salmonella

The technique used for the detection of *Salmonella* is established on the chemically modified nucleic acids to target *Salmonella*. In this method, we use the universal nuclease activity of *Salmonella* as a biomarker. For the detection strategy, chemically modified oligonucleotides which are flanked at the 5' end and at the 3' end a fluorophore (FAM) and a quencher (TQ2) are used. FAM acts as a reporter molecule, the degradation of the oligonucleotide's probes through nuclease, and the FAM records the activity through fluorescence measurements (Hernandez et al. 2014) (Fig. 3).

3. Advancement in Treatment

Salmonella species are increasingly resistant to conventional antibiotics as well as complementary therapies (Crump et al. 2015). *Salmonella* and other dangerous bacterial species are becoming increasingly resistant to antibiotics for a variety of reasons, including the overuse of antibiotics due to easy access in some countries (Medalla et al. 2016). Along with poor cleanliness habits, the use of antibiotics to protect crops and promote animal growth in animal husbandry has also contributed to the overuse of antibiotics and resulting in the rise in resistance (Nami et al. 2015; Sabtu et al. 2015; Medalla et al. 2016).

4. Use of yeast as a Prophylaxis

The recent advances in the treatment of *Salmonella* is to use yeast as a preventative agent against typhoid, paratyphoid, and NTS (non-typhoid *Salmonella*). The yeast functioned as a probiotic and its strain *S. boulardii* is most commonly used (Kelesidis and Pothoulakis 2012; Rajkowska and Kunicka-Styczynska 2012; Tomicic et al. 2016). It is also being discussed in many studies which provide knowledge about the effectiveness of different species and strains of yeast (Palma et al. 2015). One of the promising benefits of using yeast as a probiotic is that they are not targeted by the host cells as compared to the bacterial cell (Martins et al. 2009). The yeast functioned as a protective layer for the intestinal mucosa and protected it from the damage caused by pathogens, allergies, and toxins (Guttman et al. 2009). Different studies revealed that *S. boulardii* performs its function by inhibiting the production of pro-inflammatory cytokines (IL-8), and the activation of MAP kinases stops for example Erk 1/2 and JNK/SAPK. The preservation and protection of tight junctions were attributed to the *S. boulardii* anti-inflammatory factor (SAIF). It also generates proteases, which degrade the toxins created by pathogenic bacteria (Pothoulakis et al. 2009).

Control

Salmonellosis is a foodborne ailment, so all the factors that contribute to its spread are related to food and food products (Jones 2011). *Salmonella* can hide in a variety of foods, but there are a lot of ways that help to ensure that the bacteria stay away (Fig. 4):

- Don't eat raw or barely cooked eggs or meat. Uncooked food serves as one of the major factors in the transmission of Salmonellosis. Properly cooked food decreases the chances of spreading the infection (Wegener et al. 2003).
- Pasteurization has brought a revolution in the entire food industry and at the same time, it has resulted in a significant decrease in foodborne disease spread (Barrow 1993). Its most important aim is to kill pathogens within perishable food items. So, avoid using unpasteurized food items (Maijala et al. 2005).
- Avoid splashes from raw meat onto other surfaces while washing. These splashes transfer germs onto other food, like lettuce, that will not be cooked to kill germs before eating (DeBusser et al. 2013).
- Wash raw fruits and vegetables well, and peel them if possible (Chambers and Gong 2011). Washing fruits and vegetables tend to decrease the potential transfer of harmful germs such as *Salmonella*. Wash the vegetables thoroughly under fresh running water (Tellez et al. 2012).
- Refrigerate food properly before cooking and after serving it. Refrigeration slows down the growth of many pathogens (Koutsoumanis et al. 2019). *Salmonella* requires a suitable temperature, moisture, and pH for their growth and a refrigerator seizes them to make the food pathogen-free (Revolledo et al. 2006).
- Wash your hands well with soap and warm water before and after handling food (Goldbach and Alban 2006). Washing hands ensure the elimination of all types of pathogens and thus ensures quality health (Sundström et al. 2014).
- Keep kitchen surfaces clean before preparing food on these surfaces. Don't mix cooked food with raw food. Don't use the same utensils to prepare them (Hopp et al. 1999) i.e., don't use the same knife to cut raw chicken and then to slice mushrooms and use different plates or cutting boards to slice them on (Gavin et al. 2018).
- Cook meat to its correct minimum temperature. The minimum temperature which is required to cook the food and eliminate all the pathogens should be attained to ensure its purity. As far as *Salmonella* is concerned, it is massively spread by the consumption of undercooked food. Use a food thermometer to be sure (Vanderwal 1979).
- Wash your hands with soap and water after touching animals, their toys, and bedding (Mead and Barrow 1990). Avoid eating food in an area where animals are present. Minimize contact with animals having diarrhea (Vico and Jaime 2011).

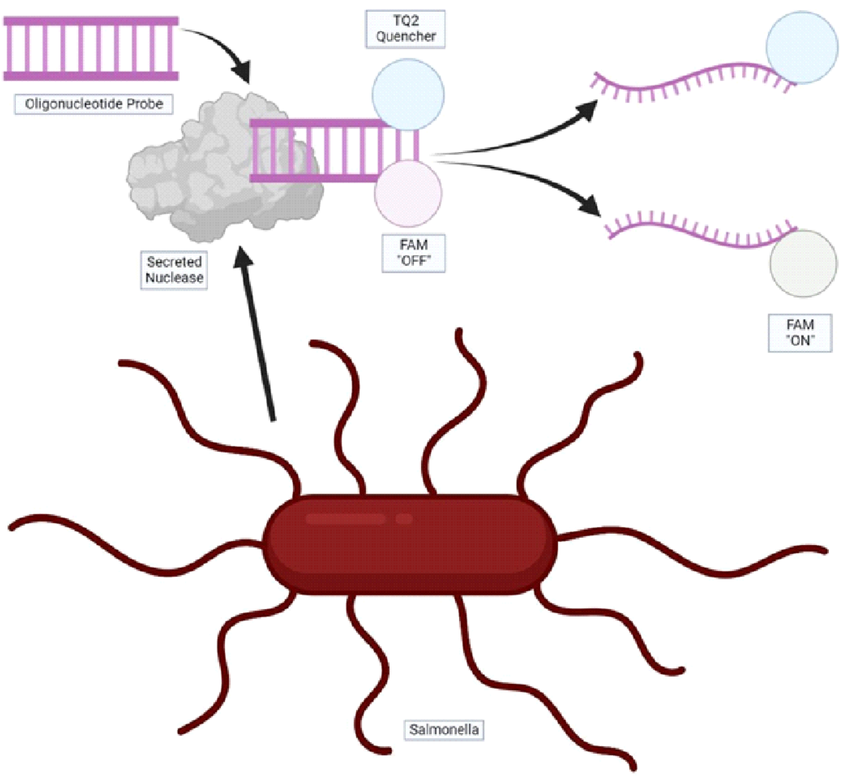

Fig. 3: The phenomenon that shows the nuclease of *Salmonella* attacks oligonucleotide and degraded it in response to which FAM records the activity.

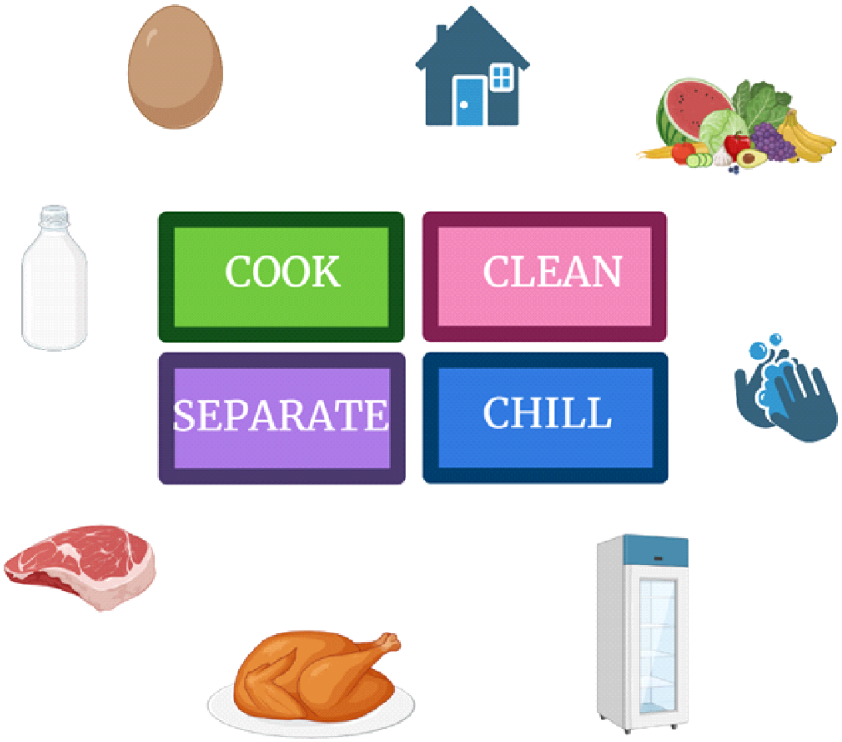

Fig. 4: Four things should be kept in mind while controlling *Salmonella*: cook meat properly, separate the raw and cooked food, refrigerate the food to slow down the production of bacteria, keep the environment clean and take care of personal hygiene

Conclusion

The rate of mortality and morbidity due to the human enteric pathogen *Salmonella* is of high magnitude even in the presence of antibiotic drugs. Due to the excessive use of antibiotics, resistance has developed in animals, so we must derive a way to get rid of antibiotics. According to the data of research it is estimated that about 2.8 billion *Salmonella*-associated diarrheas are reported annually. The major problem is the lack of a system through which the by-products of animal origin can be secured from *Salmonella* for the healthy consumption of humans. Due to antibiotic resistance in animals and humans, yeast is used as a complementary therapy for salmonellosis. Precautions should be taken while using meat, eggs, drinking water, and household objects to avoid contact with pathogens. In the control of infections caused by *Salmonella* in both humans and animals, the surveillance of the pathogen is a mandatory step.

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4

Brucellosis in Cattle and Buffaloes

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INTRODUCTION

Brucellosis is one of the most cardinal infectious and zoonotic disease in developing countries all over the world that hampers livestock production and *human* vigour (Franc et al. 2018; Dadar et al. 2020). In humans, brucellosis is known as Gibraltar fever, Undulant fever, Maltese fever, Goat fever, Crimean fever, remitting fever, and Mediterranean fever, while in cattle and buffaloes it is called Bhang's disease (or contagious abortion) (Rossetti et al. 2017; Khurana et al. 2021). Various brucella strains are responsible for this disease. Brucella is a genus of gram-negative bacteria named after Sir David Bruce (Seleem et al. 2008). Brucella has a tendency to adapt to new hosts. It causes supplementary severe complications in humans because they are a dead-end host for this pathogen (Moreno 2014). Almost 50,000 expositions of disease transmission from animals were investigated in humans around the world each year (Pappas et al. 2006). Brucellosis in bovines was first declared in Zimbabwe (Africa) in 1906 (Kiros et al. 2016). Brucellosis in cattle and buffaloes was eradicated in Ireland (1980) (Khurana et al. 2021). It is still present in India due to its lack of awareness (Machavarapu et al. 2019). In China, the ubiquity of bovine brucellosis was 1.9% (Khurana et al. 2021). 6.3% of RBPT (Rose Bengal plate test) samples and 18.6% of herds are positive for bovine brucellosis in Pakistan (Ali et al. 2017). If proper control measures and vaccines are not adopted, then the ubiquity of disease will be higher because of a poor immune system (Ali et al. 2014; Dorneles et al. 2015). Only the Brucella vaccine cannot eradicate this disease. Along with the vaccine, undeniable preventive measures should be practiced (Seleem et al. 2010; Awah-Ndukum et al. 2018). Successful control procedures should be adopted to control brucellosis such as culling. Culling prevents disease transmission and inspection (Rahman et al. 2011; Durrani et al. 2020). Certain countries have restricted brucellosis by adopting control measures such as valid diagnostic appliances, smooth live immunization, and mass vaccination of huge populations (Moreno 2014). However, the elimination program is very costly, but Americans consume $1 on this program and save $7. In the USA, the elimination program's cost was $3.5 billion in 1934-1997 but in 1952, the price of abortion and decreased milk yield was about $600 million. The prevalence of brucellosis in humans varies between 0.03 and 160 per million people. In America, a $600 million loss is recorded due to brucellosis (Acha and Szyfres 2003; Pappas et al. 2006; Sriranganathan et al. 2009). In Pakistan, losses due to brucellosis, in dairy cattle were estimated at $3.4 billion and in India it was $58.8 million due to effective surveillance programs (Jamil et al. 2021). Around the world, economic losses effect the animal productivity and public health (Acha and Szyfres 2003; Pappas et al. 2006 Sriranganathan et al. 2009).

Brucella Organisms

Brucella species are pathogenic, facultative, non-motile, gram-negative coccobacillus, having a size of 0.6-1.5 µm length, 0.5-0.7 µm diameter that belongs to the menage of Brucellaceae (Moreno 2014). The menage Bruceallaceae consists of Brucella and 6 other genera (Leclercq et al. 2020). Recently, 12 species of Brucella have been identified (Whatmore et al. 2016). *B. abortus*, *B. suis*, *B. melitensis*, *B. canis*, *B. neotomae* and *B. ovis* are pathogenic Brucella species that affect cattle, pigs, goats, and sheep, as well as dogs, small rodents, desert rats, and rams, respectively. *B. abortus*, *B. melitensis, and B. suis* affect humans (De Bolle et al. 2015). These Brucella species affect wild and domestic animals (Lindahl et al. 2014; Wareth et al. 2014). In marine animals, 2 more species are reported: *B. inopinata* (isolated from humans) and *B. papionis* (isolated from baboons) (Olsen and Palmer 2014; Whatmore et al. 2014).

From frogs, 36 brucella species are isolated. Brucella also has biovars, 7 for *B. abortus*, 5 for *B. suis*, and 3 for *B. melitensis*. The remaining species have not been classified into biovars yet. Brucella belongs to the Proteobacteria phylum. It is gram negative, nitrate reductase and have partial acid fast, urease and catalase activity. This organism has the ability to survive

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in freezing conditions. It has high sensitivity to most common disinfectants. It can survive in cool and moist environment. Pasteurization can kill bacteria. So, pasteurization of milk is necessary for the prevention of bacteria. These organisms are non-motile and do not have any genes for flagella (Olsen and Palmer 2014; Scholz et al. 2016). The nomenclature of *Brucella* spp. Is based on the principal host species (Khurana et al. 2021). Entire brucella species have same genome atlas and magnitude (Sriranganathan et al. 2009). The genome size of brucella is 3.29Mb, which is divided into 2 chromosomes. Chromosome l has a genome size of 2.11 Mb and a G+C placid of 57.2%, while chromosome ll has an genome size of 1.18 Mb and a G+C placid of 57.3%. An allele sequence SKN13 of *B. abortus* was withdrawn from cattle's placenta in India, which is very helpful for comparative genetic data analysis. In some species, some virulence genes are absent (plasmids, pili, capsules) (Chauhan et al. 2016). Sankarasubramanian et al. (2017) explained that *B. abortus* has 143 vintage-specific single nucleotide polymorphisms (SNPs) in 311 alleles. Of these, 141 are significant SNPs. While in *B. melitensis*, from 132 alleles, there are 383 vintage-specific single nucleotide polymorphisms and of these, 379 are significant SNPs. These SNPs can bias host adaptation. Brucella's outer membrane is related to gram-negative bacteria. It has A and M LPS surface antigens. In *B. abortus* and *B. suis*, A dander is the main antigen, while in *B. melitensis*, M is the main dander. LPS is the main virulence factor in brucella. For diagnostic tests, outer membrane amino acids (proteins) are very important (Khurana et al. 2021).

Significance in Livestock

Brucellosis is still neglected in many areas, which causes serious health problems and effects the economy of livestock (Santos et al. 2013; Singh et al. 2015). In livestock, Brucella causes abortion and infertility (in both sexes), which is the major reason for economic loss (Sulima and Venkataraman 2010; Deka et al. 2018; Franc et al. 2018). Abortions, decreased milk production, obstacles in animal transport and marketing, decreased productive capacity, increased veterinary drug costs, control program expenses, missed reproductive cycles, and low market price are all major economic losses (Georgios et al. 2005; Blasco and Molina-Flores 2011; Dadar et al. 2020). If abortion occurs in the cow once in its life, then the infection remains asymptomatic in that animal throughout its life (Godfroid et al. 2010). The Brucellosis threat is increased due to animals' mixed farming (cows, buffaloes, sheep, and goats) (El-Wahab et al. 2019).

Pakistan is an Asian country in which agriculture plays a very vital role in the economy. In agriculture, the livestock sector plays a very important role. According to the economic survey of Pakistan in 2019, Pakistan has 90.8 million large ruminants, 109.4 million small ruminants, and 1.1 million camel population. The contribution of livestock to the national GDP is 11.7 percent, and in agriculture, it is 60.6 percent (2019-2020). In Pakistan, a major population of large ruminants is allocated around the areas of Punjab and Sindh, while a major distribution of sheep and goats is found in AJK, Baluchistan, KPK, and Gilgit-Baltistan. Pakistan ranked 4th in milk production in 2019 and its milk production is 61.7 million tonnes (Jamil et al. 2021). Nowadays, the cost of medication has decreased due to safety practices. Unintentionally, exposure of milk to *B. abortus* causes major economic losses and increases the human brucellosis threat (Singh et al. 2015). In Pakistan, exotic cattle, buffaloes, and crossbred cattle have higher brucellosis acceptance. Sahiwal cows have more resistance to brucellosis. Males have a lower occurrence of brucellosis than females (Jamil et al. 2021).

Punjab has the largest human and animal population. Rivers and the monsoon season (rain) provide excellent agricultural yield opportunities (July, August, and September). Due to traditional and best farming methods and the best veterinary diagnosis and inspection system, weather remains extreme from extreme winter (-37 °C) to extreme summer (46-54 °C). A high number of cases of brucellosis have been reported in Punjab. Brucellosis cases are highly variable and their variability depends upon diagnostic tests, environmental conditions, animal type and farming techniques (0–69% in bovines, 4.4–20% in equines, and 7–35% in sheep and goats). High brucellosis prevalence occurs in confined farming systems (Jamil et al. 2021). Khan et al. (2021) conducted an experiment with 300 cattle from Punjab (Sargodha, Sahiwal, and Chiniot). They take their blood samples for serum analysis through different tests (RBPT, iELISA, RT-PCR). The result indicates that 12.7% of cattle are seropositive. All PT-PCR-tested samples are positive for Brucella infection. Sindh ranks second in terms of human and animal (livestock) population and third in terms of area. In Sindh, Brucellosis cases are usually seen in large ruminants and camels but not seen in small ruminants. Pathogen levels in camels range from 12-32.4%, while in cattle they range from 17-25%. Khyber Pakhtunkhwa comes in 3rd place in livestock and human's population. In this province, bovines are found in larger numbers than small ruminants, but brucellosis is seen in both of these i.e., 0–13% in cattle and 3.2-16.7 % in small ruminants. Balochistan has the least population and the largest area of any province in Pakistan. In Balochistan, small ruminant cases are more commonly reported than large ruminant cases. Seroprevalence in bovines is 0.3-6%, while in small ruminants it is 2-2.7%. In Islamabad, brucellosis cases have been reported in large ruminants (1.6-8.3%) and small ruminants (2.2-13%). In Gilgit-Baltistan, brucellosis is reported in wild animals and in cattle (10.9%). In Azad Jamu and Kashmir, brucellosis is usually reported in goats at 13.3%. Almost 17 field types of *B. abortus* have been reported from bovines (Punjab) and one isolate of *B. melitensis* from goats (KPK). Confirmation of *B. melitensis* has been done by polymerase chain reaction (real-time PCR). Moreover, detection of *B. abortus* has been done by real-time PCR in canines, camels, small ruminants, and horses.

Lipopolysaccharide (LPS) antibodies of brucella have not been detected by any polymerase chain reaction. Brucellosis is also transmitted in animals genetically. According to one theory, an allele (nramp1) can decrease the occurrence of brucellosis in cows (sahiwal). On farms, the occurrence of brucellosis in females is more due to increasing A.I practices. The risk of brucellosis increases with age in situations such as foetal membrane retention, abortion, herd size, environment, management and the introduction of carrier animals into the herd (Ali et al. 2014; Jamil et al. 2021).

Zoonotic Importance

Zoonotic diseases are those diseases that can be transferred from animals (vertebrate) to humans and brucellosis is considered as a zoonotic infection (Rahman et al. 2020). In central Asia and Middle East, brucellosis in human is increasing day by day (Khurana et al. 2021). Human brucellosis occurs mostly in those areas where this disease is endemic, or humans come back from endemic areas (Hull and Schumaker 2018). Brucella can spread by direct and indirect contact to another susceptible host (Moreno 2014). Uterus (brucella vitiated animal), aborted fetus, infected bulla, uterine discharge, placental membranes and contaminated feed or water are the main sources of dissemination (Acha and Szyfres 2001; Addis 2015; Dadar et al. 2020). Due to cross-species dissemination, its host range is very vast (Horizontal dissemination) (Moreno 2014). Through milk and milk products (butter, ice-cream, cheese, whey and yogurt) *B. abortus* can easily be transmitted in humans (Dhanashekar et al. 2012). Abattoir workers, veterinarians, meat inspectors, farmers, butchers, animal keeper, and lab workers have greater chances to get exposed by the infection. Mostly those countries are at risk where pasteurization is not used properly and hygienic practices of animal husbandry is very low (Addis 2015). Due to absence of proper administration, poor hygiene, deficient of sanitation, intensive farming and poor political will, brucellosis spread is very high (Pal et al. 2017). If accidental utilization of live vaccines such as Strain 9 of *B. abortus* and Rev.1of *B. melitensis* occur, then it can cause infections in humans (Addis 2015). *B. melitensis* is considered to be highly endemic globally due to its re-emergence (Gwida et al. 2010). Its zoonotic importance increases due to increase global dealings of animal products, enlarge international tours, rapid deforestation, urbanization, migrating animal husbandry and unendurable development (Bayeleyegn 2007). Firm surveillance and restriction initiatives of milk pasteurization and dairy by-products could deplete the contingency of brucellosis in humans (Moreno 2014; Mailles et al. 2016; Dadar et al. 2020). Signs of human brucellosis are depression, chills, undulant fever, night sweats, nervousness, constipation, weight loss, joint pain, lack of appetite, myalgia, insomnia, loss of weight, inflammation of reproductive organs and some other body parts (epididymis, brain, spinal cord, vertebra, testes, bones and prostate gland) and sexual impotence (Acha and Szyfres 2003; Kochar et al. 2007; Mantur and Amarnath 2008; Kiros et al. 2016). Brucella affects all age group of humans and if brucellosis is not detected at the proper time, it can become chronic in nature (Addis 2015).

Pathogenesis

The main pathogenic factors in brucellosis are guanine monophosphate, lipopolysaccharide, adenine monophosphate, 42-KDa protein and urease. Brucella genetic material is lacking certain pathogenic genes that are encoded for capsules, plasmids, exotoxins and pili (Seleem et al. 2008). Their modes of transmission are wounds, feed intake, inhalation, and eyelid. After entering the host body, *B. abortus* starts multiplication in phagocytic cells (macrophagic and dendritic cells). When a female becomes pregnant, the *B. abortus*, through circulation, enters the blastocytic cells and mammary glands. It enters the placenta by blood (Moreno 2014; de Figueiredo et al. 2015) and transfers to the fetus. Allantoic fluid increases the growth of bacteria; thus, it makes the reproductive site as a predilection site. If the erythritol levels increases from the 5th month of pregnancy, it may cause abortion. After ulcer formation and breakage of cells in the chorio-allantoic membrane, erythrophagocytic trophoblasts are found in the placentome. Damage in placentome, due to bacteria and stress, causes abortion (Khurana et al. 2021). Fig. 1 illustrate the pathogenesis of brucellosis (Acha and Szyfres 2001; Neta et al. 2010; Poester et al. 2013; Roset et al. 2014; Kiros et al. 2016; Khan et al. 2021; Khurana et al. 2021). Except in pregnant animals, this bacteria spreads in the environment through secretions and metabolic wastes. From one study it was analyzed that changes are seen in adenosine deaminase pursuit and the oxidative stress levels in serologically positive brucellosis cows in Brazil. Adenosine deaminase and catalase activity are decreased, whereas oxidative stress levels are increased in *B. abortus* animals. As brucella species are intracellular pathogens, they can live within neutrophils and monocytes (phagocytic cells) by different escape mechanisms and changes into acute, chronic and carrier forms (Khurana et al. 2021).

Clinical Signs

Clinical signs due to *B. abortus* include retention of fetal membranes, abortion (30–80% in vulnerable animals), birth of weak young ones, inflammation of the uterus, low fertility, fibrinous pleuritis, chronic mastitis, interstitial pneumonia, swelling of joints, edema in inter-cotyledonary placenta, leathery texture placenta and necrosis of cotyledons (Neta et al. 2010; Kiros et al. 2016). It mostly infects the reproductive system. Calves can show signs of infection at a mature age if infected with *B. abortus*. *B. abortus* incubation period varies from a few weeks to months (Kiros et al. 2016; Abdisa 2018). In male animals, *B. abortus* causes inflammation of the testes (orchitis), necrosis of the testis, inflammation of the epididymis (epididymitis), hygroma (in chronic cases),

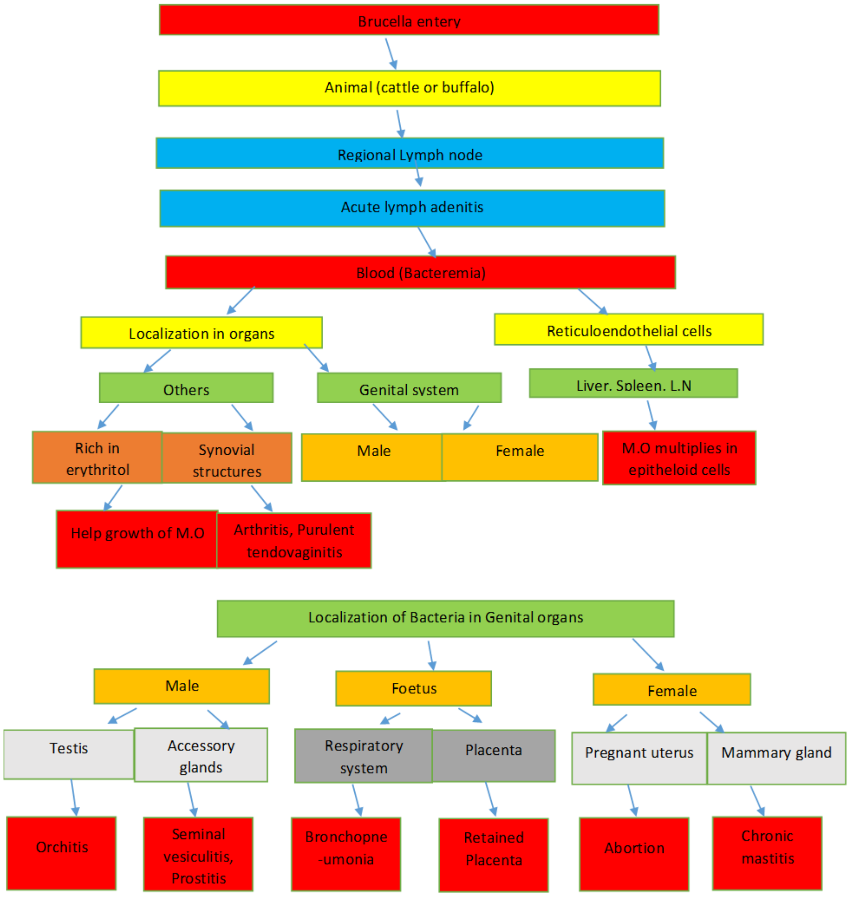

Fig. 1: Pathogenesis of Brucellosis

cervical bursitis, seminal vesiculitis and smaller testis than normal. Soft lesions containing thin and watery purulent exudate may soften the testis at times (Kiros et al. 2016; de Macedo et al. 2019).

Clinical Pathology

In lymphatic tissues and organs, small areas of inflammation are seen that are called granulomas. In the general stage of infection, pathogen is localized in tissues persistently. In the cases of abortion (female) and infertility (male), *Brucella* is seen in the reproductive tracts (Enright 1990; Acha and Szyfres 2001; Neta et al. 2010). An encroached bovine uterus with necrotic placentitis causes the fetus to die, and then abortion occurs. If abortion does not occur, then this kind of uterus and placenta lead to the birth of the infected calf. The cotyledons are swollen and enveloped by a yellowish or tenacious brown ooze. The inter-cotyledonary regions are condensed, cloudy and leathry, from which oozing of reddish fluid occurs. From a miscarried fetus, the body cavity is enlarged due to fluid accumulation and enlargement of organs (liver, spleen). From 4 months to a year, the fetus may be hairless (Khurana et al. 2021). In the lungs, pneumonitis is seen. Sometimes, fibrinous granulation and congestion may also occur. In several cases, infiltration of bronchioles, perialveolar tissue and peribronchial tissues is noted. Cobblestone bruises on the lungs are the main characteristic sign of brucellosis. In the mammary glands, macrophages accumulate in the interstitial spaces (Stableforth and Galloway 1959; Neta et al. 2010).

Bacterial Cell Cycle and Chromosomal Replication

Brucella growth is asymmetric and starts from one pole of the division site, but its lateral replication cannot be seen yet (Brown et al. 2012). The asymmetric replication of bacteria in intra-cellular nuclei is not properly seen but unipolar replication can seen by Texas Red Succinimidyl ester (TRSE). The amines on the bacterial surface are covalently bound by TRSE and this label is stationary. That's why replication (growth) is seen by increasing fragments of the cell body (unlabelled) (Khurana et al. 2021). Fluorescent d-AA (amino acid) can form spots on the growing region of peptidoglycan. Until now, it has not been applied to *B. abortus*. Polar growth of *B. abortus* is associated with the polar system's location. This system produces Cgs (cyclic-beta-1, 2-glucan), which has 17 to 25 glucose remnants (cyclic polymer) that are essential for pathogenicity and as an osmotic shock resistant. This polymer Cgs also disrupts the cholesterol-rich plasma

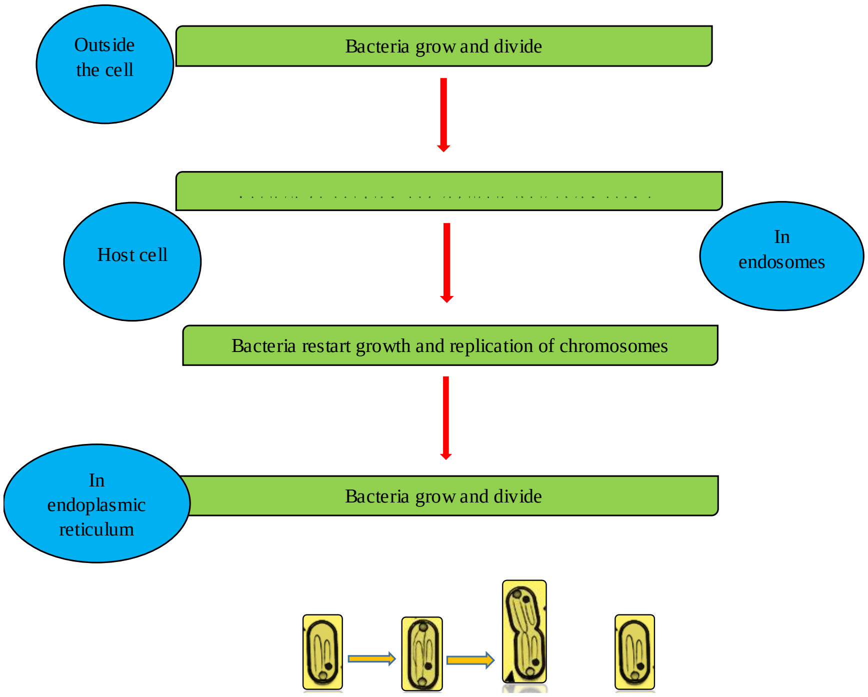

Fig. 2: Integration of Brucella's cell cycle with intracellular piracy

membrane domains of the host cell. Afterward, this polymer (Cgs) is transported (Cgt) into periplasmic space (Bundle et al. 1988; Arellano-Reynoso et al. 2005; Roset et al. 2014; Guidolin et al. 2015; Kuru et al. 2015). In the non-polar growth of bacteria, Cgs diffuses into the periplasm by Cgm. This complex (Cgs-Cgt) shows that other periplasmic complexes are also manufactured at the pole. In fact, peptidoglycan synthesis mechanisms and LPS export processes can be started at the division site's growth pole (De Bolle et al. 2015). Because of its growth and division outside the host, G1 bacteria have a high chance of host cell entry. After entering the host cells, these bacteria remain in endosomes. In endosomes, these bacteria remain in the G1 phase for many hours (depending on the host cell type). During this time, they do not grow and replicate. After the beginning of DNA replication, bacteria convert into their rBCV (replicative phase). This stage (replicative stage) is found in Endoplasmic reticulum. They restart the expansion and reproduction of chromosome No. 1 (chr.1). Then eBCV converts into rBCV. For this conversion, Vir B is required. Vir B production is influenced by the circumstances that occur in eBCV (acidic pH, starvation), as shown in Fig. 2 (De Bolle et al. 2015).

B. abortus has two chromosomes, Chr.I and Chr.II. However, the magnitudes and numbers of chromosomes differ between *Brucella* species. Chromosome 1 (chr. I) is long (2 Mb), discoid, and the place where its replication starts is detected 115kb from dnaA gene (genes that are made up of DNA), near to the parAB operon which is called orill. Chromosome II (chr. II) is smaller than chromosome I (1.2 MB). It is called a chromid (De Bolle et al. 2015). Both chromosomes have similar G+C content, but their duplication and division processes are different from each other. Chromosome II has genes that code for a network called the RepABC system. In RepABC system, Rep C initiates the DNA replication, but the other two (Rep A and Rep B) isolate the duplicated synchronization origin of orill (chr. II) that is thought to be placed inside the genes of repABC system (Pinto et al. 2012). While *B. suis* has two fused chromosomes, and when a cell divides from *B. abortus*, it gives rise to two daughter cells (De Bolle et al. 2015). This process occurs in 210 minutes (in a rich medium). This division occurs in G_2-phase (175 minutes after the initiation of this separation), whereas chromosome replication starts in S-phase (175 minutes after the initiation of this separation). Orill replication and segregation starts after 105 minutes of this division. And constriction begins 35 minutes prior to this division. These types of bacteria (constricting) are called pre-divisional bacteria, as shown in Fig. 3 (Hallez et al. 2007).

Diagnosis

For diagnosis of Brucellosis epidemiological patterns and history plays a very important role. There are different methods for diagnosis of brucellosis as illustrated in Fig. 4 (Lucero et al. 2003; O'Leary et al. 2006; Glynn and Lynn 2008; OIE 2009; Godfroid et al. 2010; Ali et al. 2014; Durrani et al. 2015; Arif et al. 2018; Khurana et al. 2021).

For isolation, bacteria can be collected from the uterus, milk, aborted fetus, iliac and supra-mammary lymph nodes, and spleen. Other body parts, such as the eyes, bones, brain, and joints, can also become infected. In male animals, bacteria can be obtained from genital organs and lymph nodes. In the acute phase of brucellosis, bacteria can be obtained from the semen as well. In the chronic phase, the bacteria level

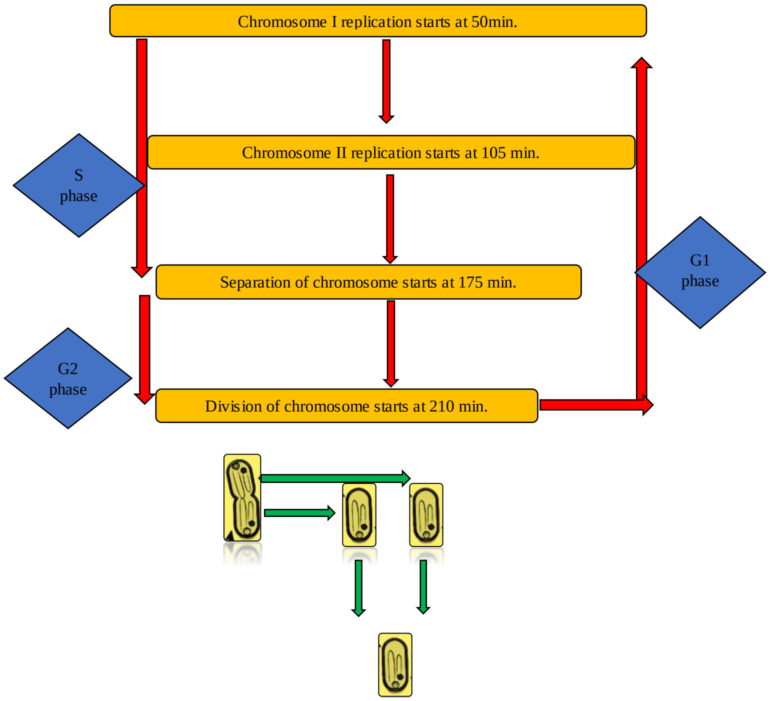
Chromosome I replication starts at 50min.
S phase
Chromosome II replication starts at 105 min.
G1 phase
Separation of chromosome starts at 175 min.
G2 phase
Division of chromosome starts at 210 min.

Fig. 3: Cell cycle of *B. abortus*

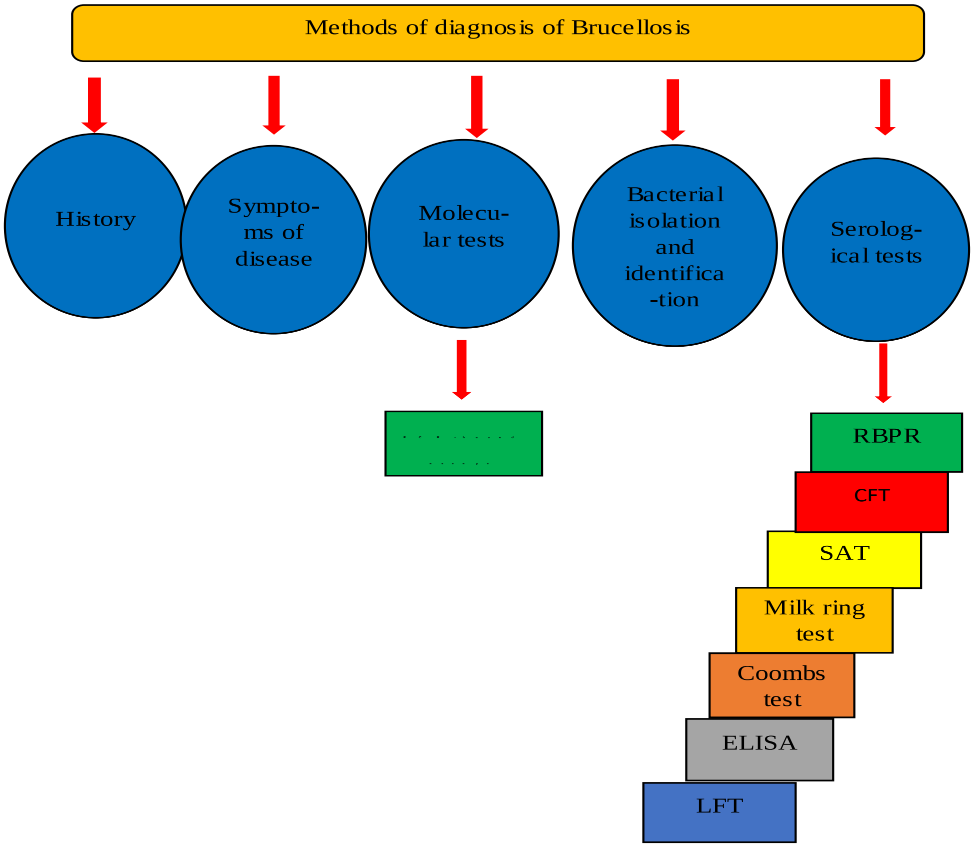

Fig. 4: Methods of diagnosis of brucellosis
PCR=Polymerase chain reaction, RBPT =Rose Bengal Plate Test, SAT=Standard Plate Agglutination Test, CFT= Complement Fiation Test, ELISA= Enzyme-linked immunosorbent assay, LFT= Lateral Flow Assay

decreases in excretion. The media that are used for brucella are TSA (trypticase-soya agar) and Columbia agar. *B. abortus* needs CO_2 and sera for development. A polymerase chain reaction assay (PCR) is a fast diagnostic procedure (Khurana et al. 2021). This procedure is also useful for diagnosis of biovars, analysis of treatment potency, and relapsing brucellosis (Christopher 2010). Real-time polymerase chain reaction (PCR) is used for an accurate diagnosis of *B. abortus* (Redkar et al. 2001). This PCR observes different regions of *Brucella* genetic material (16S rRNA, IS711, and 31-KDA). An IS711-based PCR is used for the identification of *Brucella* from lympathic tissues, milk, and blood (O'Leary et al. 2006). PCR-based methods differentiate between the S19 vaccine and toxic strains of *B. abortus* (Kaynak-onurdag et al. 2016). IHC (immunohistochemistry) was used for the confirmative analysis of brucellosis in placental cotyledons and aborted tissues (Safari et al. 2019). Serological tests are used in eradication, inspection, and control strategies worldwide (Lucero et al. 2003). RBPT is useful for validation of swelling in the scrotum (hydrocele), neuro-brucellosis, swelling of joints (arthritis), inflammation of the epididymis (epididymitis), and swelling of the testis (epididymitis) (Mantur et al. 2006). CFT (complement fixation test) is used for exact detection of antibodies (IgM, IgG1). It can also be used in the serum of vaccinated (PB51) bovines. SAT (standard tube agglutination test) is an economical test. ELISA (enzyme linked immune-sorbent assay) is used for *Brucella* antigen sensing. It is considered the most accurate test (100% sensitivity and 99% specificity) for *Brucella* antigen. CFT is also best for persistent brucellosis. ELISA gives better results than acute brucellosis in conventional assays. Indirect ELISA is helpful in clinical brucellosis detection and is more accurate than SAT. It is very sensitive for CNS brucellosis detection and measures antibody (IgG, IgM, or IgA) levels in serum. For milking animals, indirect ELISA and milk ring tests are mostly used. The Coombs test and Burnet's intradermal tests are used to observe the condition of intolerance of contaminated essence to *B. abortus* (Khurana et al. 2021). Rose Bengal plate test (RBPT) gives most accurate detection (99%-99.5%). RBPT is an economical test used for sera screening (Arif et al. 2018).

Treatment

Utilization of antibiotics (streptomycin, tetracyclines, aureomycin and terramycin) can cause reduction in abortions in infected cattle. These antibiotics may be used in combination or alone. Due to cost of treatment, existence of antibiotic residuses in milk and treatment failure, this therapy is not satisfactory for bovine brucellosis. Oxytetracycline alone or with streptomycin is successful because it decreases the scattering of bacteria and terminating the signs of brucellosis at the time of calving. Oxytetracyline 20 mg/kg, I/M for 14 days (every 3rd day) along with streptomycin 20mg/kg, I/M, for 7 days (every day) may be used. Effective management is also necessary for bovine brucellosis treatment (Singh et al. 2014).

Control

S19 (live attenuated vaccine) and RB 51 (rough, rifampicin-resistant strain) vaccines are mostly used in bovine brucellosis. Under field conditions, S19 gives good results in cattle (80-95%). It provides immunity for entire life span of the cattle, if cattle is immunized in early age. In male calves, S19 does not give good results. If female calves are vaccinated with S19 then at mature age (heifers) abortion does not occur as no bacteria found in their reproductive system. Being infective for humans, S19 vaccine requires special preventive measures before use (gloves, protective glasses, masks, and sleeve coats). RB51 vaccine is best in cattle for control of abortion. Calves are protected against *B. abortus* sepsis and abortion, if they are immunized with RB51 (at 3, 5 and 7 months of age). It is successful in field conditions. Heifers can be immunized at the age of 10-12 months with *B. abortus* 2308 (virulent) strain. Some experiment shows that RB51 can be given in pregnancy when vaccination is not done in these cattle at early age. If cattle are vaccinated with strain 19 at early age, than RB51 can be given in pregnancy (Poester et al. 2006; Dorneles et al. 2015).

Conclusion

Brucellosis is considered as one of the most important and widespread zoonotic disease worldwide. *B. abortus*, *B. suis*, *B. melitensis*, *B. canis*, *B. neotomae*, and *B. ovis* are pathogenic *Brucella* species. The demand of milk increases with increase in population and the farmer does not know well about this disease. It is most prevalent in humans due to less adaptation of proper control strategies in Pakistan. The prevalence of this disease is on the rise owing to numerous hygienic, social, economic and cultural factors. Once entered in the host body, *B. abortus* multiplies in intracellular milieu of phagocytic cells such as macrophages and dendritic cells. When female conceives, the bacteria reach trophoblasts and the mammary gland through circulation where they multiplies to induce abortion. Brucellosis control programs mainly depends on early, accurate and precise diagnosis of the disease. It is diagnosed by history, disease symptoms, bacteriological isolation and identification, serological tests and various molecular tests. For control of this disease two vaccines, S19 and RB51 are given. For treatment combination of antibiotics are used. Main problems due to this bacteria are epididymitis, abortions, endometritis, retention of fetal membranes etc. Due to this disease major losses occur that includes animal health problems, production and effects on public health.

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5

Bacillus anthracis: A Bioterrorism Agent

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INTRODUCTION

Bioterrorism broadly describes the use of biowarfare agent (chemicals, bacteria, and viruses) to induce disease in people or even kill them and may damage crops and livestock. It is the intentional use of a pathogen or chemical to destroy the economy of a country and also affects the social life of people (De Felice et al. 2008). Bioterrorism earned great attention during the first decade of the 21st century. Definitions of bioterrorism may vary by source and have evolved over time. According to Spencer, "the use of microorganisms as weapons with devastating effects, which can be described serious physical, psychological, or economic harm to a nation" (Rose and Larrimore 2002). It highlights some important points including the broad spectrum of microbes must be considered along with their physical, psychological and economic impact. In addition, Spencer describes the term agroterrorism: " any agent which is vulnerable to livestock and crops" (Tegnell et al. 2003). Various types of biological agents include viruses, bacteria, toxins and chemical agents (Fig. 1). Broadly biological agents have been grouped into three categories as represented in Fig. 2 (Jernigan et al. 2001).

Epidemiology of Bioterrorism

Unexceptional disease outbreaks may indicate a bioterrorism attack including exotic diseases i.e. plague, inhalation anthrax, typhoid tularemia, smallpox, hemorrhagic fever, and typhoid fever suggest the intentional dissemination (Mayer et al. 2001). Bioterrorism disease has relatively nonspecific characteristics, therefore, epidemiologists try to understand the cause of the disease. Unexpected regional or transient patterns are sign that disease outbreaks are not occurring naturally. For example, all cases of anthrax around Sverdlovsk were found active within 4 km of the military headquarters, indicating military territory was the target site or source of the outbreak. All cases were reported during the same era, suggesting the uncommon outbreak of a single biological agent (Bush et al. 2001). Atypical clinical symptoms may indicate an abnormal route of infection i.e., inhalation of anthrax. Unusually high incidence rates may occur in people exposed to a particular area or if people are living in protected areas, especially if multiple dotted source outbreaks occur at the same time (Bravata et al. 2004). If it occurs, it may indicate an intentional infection. A very high number of cases, and failure to identify a single source of infection, divert the scientists' intentions to associations with food contamination (Mondy et al. 2003).

History of Biological Agents

As early as 600 BC, infectious diseases were believed to have the potential to harm both civilians and armies. The opponent was utterly destroyed by the crude use of filth, animal carcasses, and pathogens (Nofal et al. 2021). Through the various European wars, the American Civil War, and even into the 20th century, the way of poisoning the opponent army's wells and other water sources remained continued. Even Middle Ages military leaders found that victims of infectious diseases may turn into weapons. A brief history of the use of biological agents is shown in Fig. 3 (Lashley 2004).

Bacillus anthracis: A Biological Agent

Introduction

A German physician Robert Koch discovered *Bacillus anthracis* (*B. anthracis*) in 1876. *B. anthracis* is an etiological

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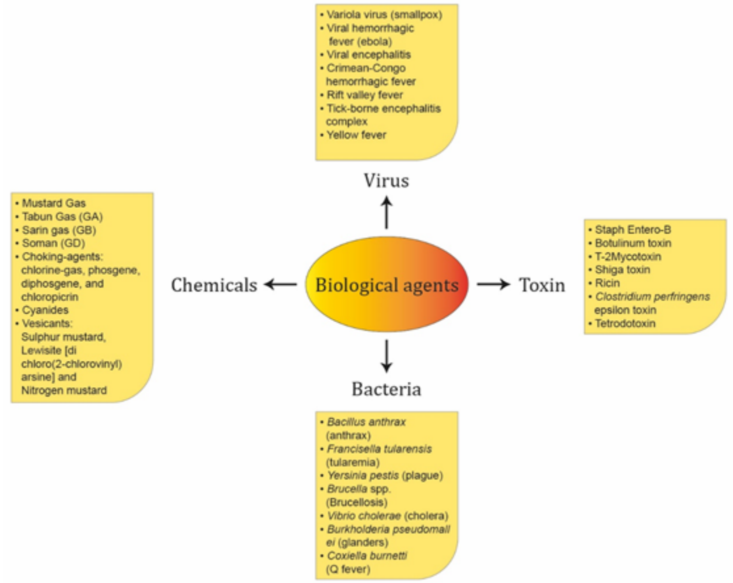
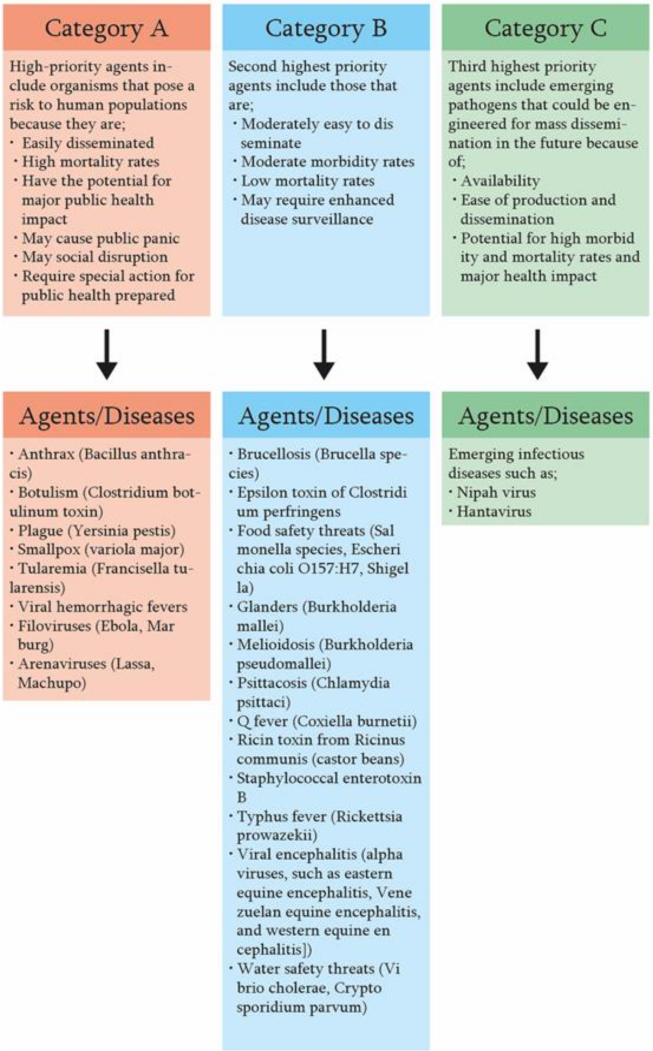

• Variola virus (smallpox)
• Viral hemorrhagic fever (ebola)
• Viral encephalitis
• Crimean-Congo hemorrhagic fever
• Rift valley fever
• Tick-borne encephalitis complex
• Yellow fever
Virus
• Mustard Gas
• Tabun Gas (GA)
• Sarin gas (GB)
• Soman (GD)
• Choking-agents: chlorine-gas, phosgene, diphosgene, and chloropicrin
• Cyanides
• Vesicants: Sulphur mustard, Lewisite [di chloro(2-chlorovinyl) arsine] and Nitrogen mustard
Chemicals
Biological agents
Toxin
• Staph Entero-B
• Botulinum toxin
• T-2Mycotoxin
• Shiga toxin
• Ricin
• *Clostridium perfringens* epsilon toxin
• Tetrodotoxin
Bacteria
• *Bacillus anthrax* (anthrax)
• *Francisella tularensis* (tularemia)
• *Yersinia pestis* (plague)
• *Brucella* spp. (Brucellosis)
• *Vibrio cholerae* (cholera)
• *Burkholderia pseudomallei* (glanders)
• *Coxiella burnetti* (Q fever)

Fig. 1: Types of biological agents



Category A	Category B	Category C
High-priority agents include organisms that pose a risk to human populations because they are; · Easily disseminated · High mortality rates · Have the potential for major public health impact · May cause public panic · May social disruption · Require special action for public health prepared	Second highest priority agents include those that are; · Moderately easy to dis seminate · Moderate morbidity rates · Low mortality rates · May require enhanced disease surveillance	Third highest priority agents include emerging pathogens that could be engineered for mass dissemination in the future because of; · Availability · Ease of production and dissemination · Potential for high morbid ity and mortality rates and major health impact
Agents/Diseases	**Agents/Diseases**	**Agents/Diseases**
· Anthrax (Bacillus anthracis) · Botulism (Clostridium botulinum toxin) · Plague (Yersinia pestis) · Smallpox (variola major) · Tularemia (Francisella tularensis) · Viral hemorrhagic fevers · Filoviruses (Ebola, Mar burg) · Arenaviruses (Lassa, Machupo)	· Brucellosis (Brucella species) · Epsilon toxin of Clostridi um perfringens · Food safety threats (Sal monella species, Escheri chia coli O157:H7, Shigel la) · Glanders (Burkholderia mallei) · Melioidosis (Burkholderia pseudomallei) · Psittacosis (Chlamydia psittaci) · Q fever (Coxiella burnetii) · Ricin toxin from Ricinus communis (castor beans) · Staphylococcal enterotoxin B · Typhus fever (Rickettsia prowazekii) · Viral encephalitis (alpha viruses, such as eastern equine encephalitis, Vene zuelan equine encephalitis, and western equine en cephalitis]) · Water safety threats (Vi brio cholerae, Crypto sporidium parvum)	Emerging infectious diseases such as; · Nipah virus · Hantavirus

Fig. 2: Categories of biological agents.

agent of a zoonotic disease called anthrax that occurs in herbivores and other animals and it is important for both public health and biodefence protection (Mukarati et al. 2020). The word anthrax originates from the Greek letter, "*anthracite* "which signifies Black Coal. *B. anthracis* is a gram-positive, non-motile and spore-forming bacterium. In favorable conditions, its spores can survive for up to ten years (Hueffer et al. 2020). In addition, spores have high resistivity to natural condition so it is supposed to be the most potent biological weapon agent. Biological agents are considered to be the most potent weapon used in bioterrorism since they are often inexpensive and have a broader impact than physical damage (Chateau et al. 2020). Countries with agriculture as a main source of income are more vulnerable to the attack of anthrax. The spores of *B. anthracis* enter the body through skin lesions, gastrointestinal routes and lungs. Once spores enter the body, they can replicate in the blood, can travel to lymph nodes, and can produce toxins that may even cause death. Symptoms include flu-like infection, shock, acute respiratory distress and ultimately death (Hoffmaster et al. 2006).

This bacterium has two large plasmids pXO1and pXO2, both necessary for toxicity. Plasmid pXO1 harbor the structural genes for anthrax toxin proteins and pXO2 carries genes for capsular synthesis. Even though *B. anthracis* forms a strongly monomorphic phylogeny with the *B. cereus* complex; *B. thuringiensis* and *B. cereus* strains that are genetically related to the *B. anthracis* cluster (Carlson et al. 2019). This bacterium can infect humans in three ways including cutaneous, respiratory tract and gastrointestinal tract. A most recent study also describes another way of infection which is injectable anthrax. The most severe and fatal types of the disease are gastrointestinal and inhalational anthrax. The virulence mechanism of anthrax allows the bacterium to spread to the lymph nodes and subsequently to the bloodstream leading to internal infection. In the host, *B. anthracis* has two forms i.e., vegetative and spores (Inglesby et al. 2002; Kamal et al. 2011).

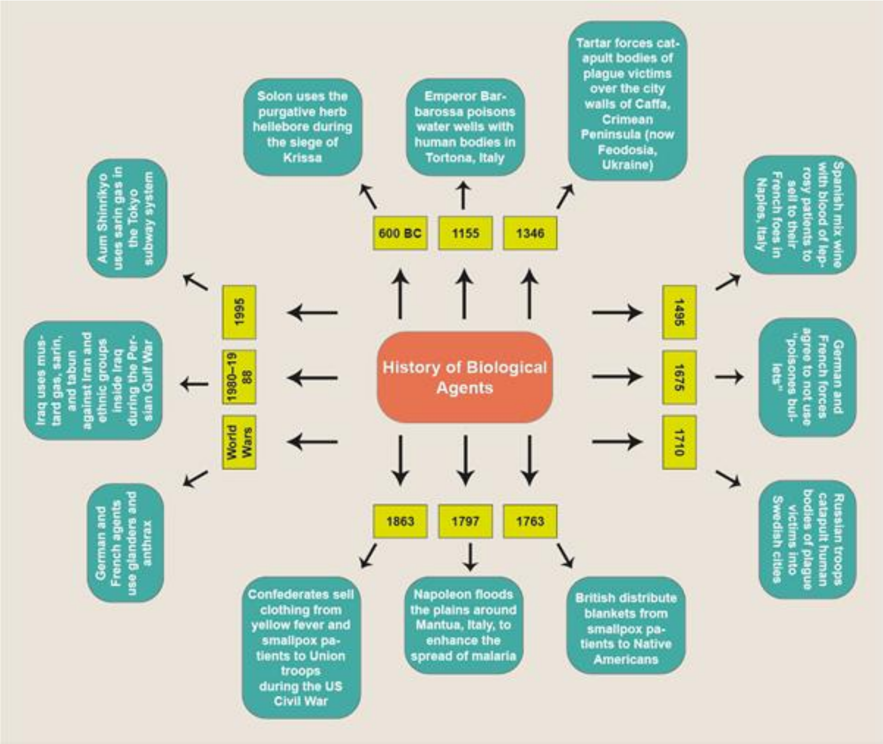

History of Biological Agents
600 BC
1155
1346
Solon uses the purgative herb hellebore during the siege of Krissa
Emperor Barbarossa poisons water wells with human bodies in Tortona, Italy
Tartar forces catapult bodies of plague victims over the city walls of Caffa, Crimean Peninsula (now Feodosia, Ukraine)
1495
1675
1710
Spanish mix wine with blood of leprosy patients to sell to their French foes in Naples, Italy
German and French forces agree to not use "poisones bullets"
Russian troops catapult human bodies of plague victims into Swedish cities
1863
1797
1763
Confederates sell clothing from yellow fever and smallpox patients to Union troops during the US Civil War
Napoleon floods the plains around Mantua, Italy, to enhance the spread of malaria
British distribute blankets from smallpox patients to Native Americans
1995
1980–1988
World Wars
Aum Shinrikyo uses sarin gas in the Tokyo subway system
Iraq uses mustard gas, sarin, and tabun against Iran and ethnic groups inside Iraq during the Persian Gulf War
German and French agents use glanders and anthrax

Fig. 3: History of use of biological agents (Lashley 2014).

This bacterium is commonly present in the soil, in the form of endospores, which can survive for many years. *B. anthracis* is categorized into pathogenic and non-pathogenic species. In addition, *B. anthracis* has two virulence factors, acid poly-γ-D-glutamic acid capsules and tri-partite toxin (Hoffmann et al. 2017). It has long been suspected of being used as a bioterrorist agent because it produces spores that can be aerosolized and proliferate the disease. During the Cold War Era and afterward as a bioterror agent, *B. anthracis* was regarded as a potential biowarfare agent (Leendertz et al. 2004). After September-October 2001 Anthrax Terrorist Attacks in the US, The U.S. Postal Service, spread spores that killed 5 people and infected 17, which lead to the identification of anthrax as a disease in 2001. As a result of these incidents, the number of scientific studies on *B. anthracis* has dramatically increased. Due to its highly pathogenic nature, spore-forming ability and high mortality rate, *B. anthracis* is known to be high-rank biological weapon (Antonation et al. 2016).

Microbiology of *B. anthracis*

B. anthracis produces large gram-positive bacilli (1-1.5 mm x 4-10 mm). This facultative anaerobe has nonmotile, encapsulated cells that are organized in chains. Swabs from infected tissue typically show evidence of encapsulation, however, this is not the case when the organism is grown in conventional laboratory culture media where it produces spores (Sharma et al. 2020). After 24 hours of incubation at 35°C, *B. anthracis* produces spherical, flat or slightly convex non-hemolytic colonies with a diameter of 2–5 mm on sheep blood agar plates (Missiakas and Schneewind 2017).

Strains of *B. anthracis*

There are three strains of *B. anthracis* namely Ames, Stern, and Vollum. Ames is a frequently examined and highly virulent strain that contains the two plasmids: pXO1 and pXO2. It was originally isolated from a dead cow in Texas in 1981. Whereas, its geographical regions are US and UK (Norris et al. 2020). First isolate of Ames strain was reported in Florida in 2001 from a victim of an anthrax attack. Stern is a toxin-producing but non-virulent strain because it carries the anthrax toxin plasmid pXO1 but lacks the encapsulated plasmid pXO2. This strain is often used in the development of animal vaccines (Pilo and Frey 2018). Vollum is a low-virulence strain used in research studies and has been found in the UK, Payne, and Zimbabwe. This strain is also used in the development of animal vaccines. Moreover, the V770 strain has also been used in the production of toxins and in various research-related studies (D'Agnillo et al. 2020).

Virulence Factors and Toxins

Toxins and autophagic capsular polypeptides, including D-glutamic acid, constitute the anthrax virulence factor. The genes for these virulence factors are on different plasmids. Anthrax toxin is composed of three proteins: EF (edema factor), PA (protective antigen) and LF (lethal factor) (Enosi Tuipulotu et al. 2021). All three parts have been studied for purification, characterization, and structural gene sequencing. On the surface of host cells, PA fragments can bind to specific receptors. Cellular proteases cleave part of the molecule to generate PA fragments that act as specific receptors for EF or LF. Host cells absorb toxins through a process known as receptor-mediated endocytosis (Aoyagi et al. 2020).

Life Cycle of *B. anthracis*

Animals become affected when they ingest anthrax endospores found in agricultural areas. Endospores find favorable conditions inside the mammalian host, such as an aqueous environment with enough nutrition, and begin to germinate. An important phase in the pathogenesis of anthrax is the transition of the spore into a vegetative cell since only the vegetative form of the bacterium can produce virulent components, such as the capsule and tripartite toxin (Antonation et al. 2016). The peptidoglycan layer and S-layers surround the poly-D glutamic acid capsule, which creates a complicated surface for *B. anthracis*. The capsule is essential for bacterial survival as it circumvents the immune system of the host. After death, the bacteria are enclosed and spread through natural orifices with blood dissemination (Hu et al. 2020). When the vegetative bacteria come into contact with oxygen, they transform into spores and re-infest the agricultural fields, where grazing animals can ingest/contact the lethal spores ultimately resulting in anthrax infection (Fig. 4).

Clinical Types of Anthrax

Depending upon the route of infection, there are three clinical types of anthrax: cutaneous (skin), gastrointestinal (ingestion), and pulmonary (inhalation of spores) (Thappa 2019). A different strain namely "injectional anthrax" has recently been discovered among European heroin injectors. Some cases of anthrax have been associated with insect bites, as some insects may spread the disease after biting an anthrax-infected animal (Berger et al. 2014).

Spores do not appear to develop in the host as the oxygen tension of the dying host is too low for sporulation or the virulence gene regulator *AtxA* inhibits sporulation (Shearer 2016). Within 3 hours of germination, spores infiltrate macrophages at the point of entry, develop into vegetative cells, propagate into tissues and begin to produce anthrax toxin (Fig. 5) (Zhang et al. 2022).

Cutaneous Anthrax

An irritating papule that looks like an insect bite is the first sign of cutaneous anthrax infection. This papule grows within a day or two and develops into ulcer with a raised, circular margin and a necrotic center (Nayak et al. 2019). Such sores often develop within 2–5 days after the spores enter the skin. After 7–10 days, a black eschar with edema surrounding it forms, leaving a scar formation after anthrax treatment. There may be swelling and enlargement of the local lymph nodes (Kasradze et al. 2018). The majorities of cutaneous anthrax infections are painless and only affect the dermis. However, when bacteria enter the bloodstream and cause bacteremia, it can spread throughout the body. However, on skin, hemorrhagic lesions may be formed (Tekin et al. 2015).

Gastrointestinal Anthrax

Gastrointestinal (GI) anthrax occurs by eating or ingesting food contaminated with anthrax spores. After intake, spores germinate and can leave sores across the body of host (Nakanwagi et al. 2020). Typically, the incubation phase lasts 3 to 7 days. Abdominal and oropharyngeal anthrax are the two forms of GI anthrax that can be identified by the lesions. In abdominal GI anthrax, lesions predominantly develop in the ileum and cecum (Owen et al. 2015).

Symptoms of abdominal gastrointestinal anthrax include nausea, bloody vomiting, diarrhea, abdominal pain, headache, loss of appetite, and severe ascites. Oropharyngeal anthrax is another type of intestinal anthrax whose lesions occur primarily in the oral cavity and resemble cutaneous anthrax. Symptoms include sore throat, difficulty in swallowing, cervical lymphadenopathy and swelling of the throat due to inflammation (Goel 2015).

Respiratory or Inhalation Anthrax

Respiratory anthrax occurs when spores of anthrax are inhaled into the lungs (also known as pulmonary anthrax). This is the most dangerous type of anthrax. The spores are taken up by alveolar macrophages and transported to the mediastinal lymph nodes. Symptoms of anthrax inhalation are similar to those of a cold or flu, initially with mild chest pain, shortness of breath, nausea, and finally fatal respiratory collapse (Azarkar and Zare Bidaki 2016). Hemorrhagic mediastinitis and pulmonary enema, unlike pneumonia, are caused by anthrax (Asgharian et al. 2016).

Injectable Anthrax

The symptoms of anthrax from injection are the same as for cutaneous anthrax, although the infection may be under the skin or deep into the muscle where the drug was injected. Sometimes the injection site may be red (Hu et al. 2020). Injectable anthrax can be difficult to diagnose because a variety of other common bacteria can infect the skin and injection site, and difficult to treat because it spreads quickly throughout the body (Fig. 5) (Mukarati et al. 2020).

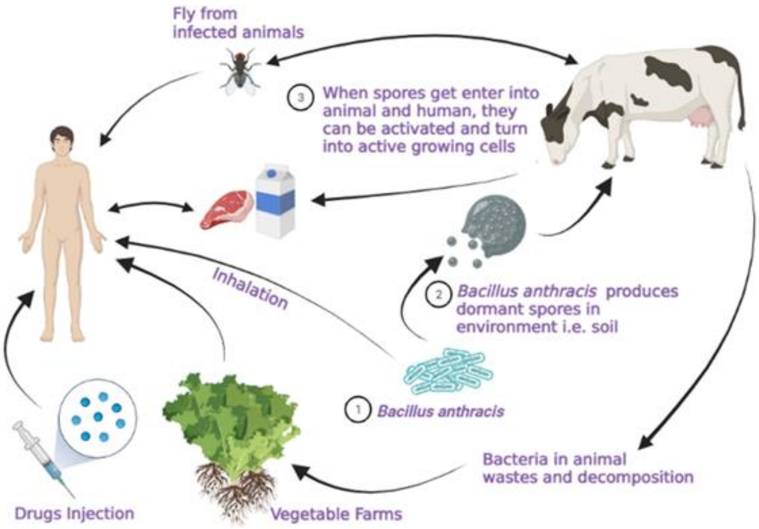

Fly from infected animals
③ When spores get enter into animal and human, they can be activated and turn into active growing cells
Inhalation
② *Bacillus anthracis* produces dormant spores in environment i.e. soil
① *Bacillus anthracis*
Bacteria in animal wastes and decomposition
Drugs Injection
Vegetable Farms

Fig. 4: Life cycle of *B. anthracis*

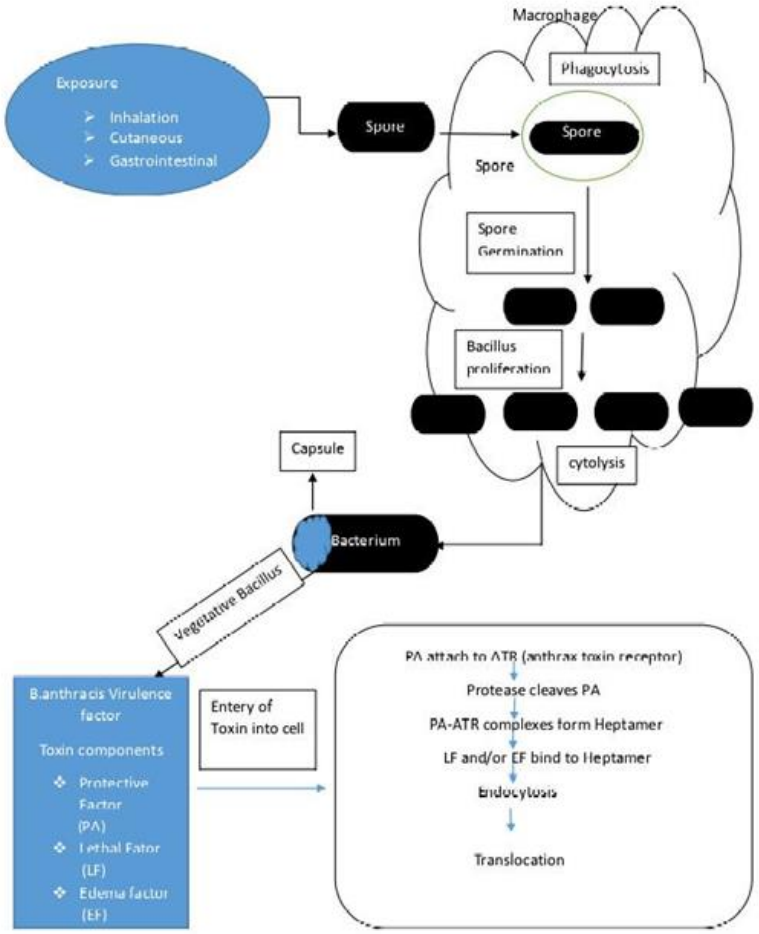


Fig. 5: Pathogenesis of *B. anthracis*

Potency of *B. anthrax* as a Bioterrorist Agent

Due to its extremely pathogenic potential and sporulation, anthrax is a top candidate for use in bioterrorist attacks. In 1970, the World Health Organization estimated that between 130,000 and 3 million individuals may die following the introduction of 100 kg of *B. anthracis* in 1993. This is just as deadly as an hydrogen bomb (Ehling-Schulz et al. 2019). According to research from animals, the human LD50 (a dose that kills 50% of infected people) is estimated to be between

2,500 and 55,000 inhaled spores. The WHO also predicted that if 50 kg of *B. anthracis* were spread over a 5-million-person urban population in 1970, it would infect 250,000 people and kill 100,000 (Berger et al. 2014). Therefore, if properly dispersed, an envelope containing 2g of powder (equal to 200 billion spores), like the one mailed to Senator Dasher in 2001, will kill thousands of individuals. From fermented cultures, it is possible to create highly pathogenic spores that, after going through a series of procedures, can be dispersed via aerosol delivery technology over a large region by an airplane (Antonation et al. 2016). Anthrax was primarily found in animals before being reported in humans. The increased significance of identifying infected individuals presents a significant challenge to public health concern. According to CDC guidelines, *B. anthracis* is enlisted in Category A high-priority diseases (Kasradze et al. 2018).

History of *Bacillus anthracis* in Different Biological Warfare

Japanese Army Units 731 and 100 purportedly experimented with the aerosolization of *B. anthracis* and the contamination of food and water supplies with enteric infections during World War II. In April 1979, a case of inhalation anthrax was recorded close to the Soviet Institute of Microbiology and Virology in Sverdlovsk, USSR, during the Cold War (Mukarati et al. 2020). The 77 confirmed cases—including 66 deaths—represent the largest known anthrax inhalation pandemic. According to more current estimates, there were 250 infected and 100 reported deaths as a result of the illegal spread. 21 cases of cutaneous anthrax from contaminated letters in 2001, 22 cases of inhalation anthrax, and 5 deaths due to anthrax in the US have been reported. Anthrax has a long history with human populations and was also popular in China and Europe (1190–1491 BC) (Goel 2015; Pilo and Frey 2018).

The fifth plague of Egypt, described in the book of Genesis (1491 BC) as an outbreak that ravaged ancient Egypt and wiped out all of the livestock, including cattle, sheep, goats, camels, horses, and donkeys may have been caused by anthrax. Anthrax in domestic and wild animals appears to be the disease that Virgil (29 BC) described in his third Georgics (collection of poems on agriculture and animal husbandry), as it was a significant agricultural disease in Europe from the 16th to the 18th centuries (Asgharian et al. 2016). In Japan, culturally identified anthrax in humans is on the national list of all reportable diseases, and medical officers must report all cases to the government. However, only 4 cases of *B. anthracis* were reported in Japan in 1990 (Takahashi et al. 2004). In August 1994, an event in Tokyo involved a man in his 80s from the Sumida district, which is adjacent to the Edo district (Kameido's site). However, there was no clear connection between this incident and that happened in July 1993. Between 1979 and 1985 more than 10,000 human cases were reported in Zimbabwe (Mayer et al. 2001). The US incident shows how human population can be affected from anthrax biological weapons (Nofal et al. 2021).

Conclusion

Anthrax has been a significant disease in the early days of microbiology, approximately 125 years ago, that halted domesticated animals and the farming industry. When this issue was identified, vaccinations were used as efficient preventative strategies. *B. anthracis* poses a major concern whether employed for terrorist or military purposes. Though treatments and preventative countermeasures are available, anthrax still poses a lethal impact on the human population. The critical steps in the present strategy are an accurate diagnosis, prompt identification, prophylactic measures, and appropriate treatment. The military and terrorist threat from anthrax inhalation is still present and may even be getting worse worldwide. Therefore, public health officials and medical professionals need to be aware of the different forms of anthrax. Pre and post exposure vaccination, and protective masks with 1 to 5 µm particle filters are important preventative measures. Though these precautionary measures may offer some protection against aerosolized *B. anthracis*, still morbidity and mortality rate are significant due to its potent bioterrorist attacks.

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6

Leptospirosis: An Overview

AUTHORS DETAIL

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INTRODUCTION

Infectious diseases transmitted via vectors or animals (Zoonotic disease) remain a leading cause of high morbidity and mortality. According to a meta-analysis, 60.3% of new infectious diseases are caused by the rapid spread of zoonotic pathogens from animals to humans and involve the interactions of humans, animals, and the environment. Various types of animals are reservoirs of zoonotic pathogens such as *Leptospira* that can circulate in different ecosystems (Urbanskas et al. 2022). Leptospirosis (LPS) is a neglected zoonotic disease recognised as the biggest public health threat around the globe with high prevalence. LPS caused by gram-negative, aerobic and slow-growing bacteria of genus *Leptospira* spp. in a group of spirochetes. The genus *Leptospira* contains numerous numbers of species which are pathogenic, intermediate and some are non-pathogenic strains based on genotypic classification as shown in Fig. 1 (Desvars et al. 2011). Serovars and serogroups are classified on a serological basis and around 300 serovars are grouped into the several numbers of serogroups (Picardeau 2017). *Leptospira* is usually 0.1 µm wide and 6-20 µm long with pointed ends bent into hook-like shapes which differentiate from other spirochetes (Samrot et al. 2021). It can grow at an optimum temperature of 28-30°C and pH of 6.8-7.6 which affects all mammalian species mostly living in a tropical climate where environmental conditions favor the prolonged survival of pathogen and transmission in poor resources and developing countries. It affects all the mammalian species and infected animals may also act as the reservoir hosts which may transmit the infection directly or indirectly depending upon the infection source (Bandara et al. 2014; Pappas et al. 2008).

The disease spread through direct contact with contagious secretions (blood, urine, aborted fetus, contaminated water, soil, feed and uterine discharge) of infected animals and distributed throughout the systematic circulation and in various organs (Hajikolaei et al. 2022; Schuller et al. 2015). These organisms enter through mucus membranes of abraded skin, vagina, nose and eyes as shown in Fig. 2 (Sykes et al. 2022).

Leptospira organisms multiply in the liver, kidney, spleen, ocular tissue, central nervous system and genital tract (Ananda et al. 2008). This infection infects humans as well as animals that impact production mainly after heavy rains (Galarde-López et al. 2021; Sud 2021; Haake and Levett 2015). The kidney and liver are the main targets during both acute and chronic phases. In these organs, they can multiply and survive for months (Haake and Levett 2015).

Population groups who are at high risk of getting infection include veterinarians, breeders, dairy farmers, meat workers and laboratory employees. However, indirect exposure to the environment (overcrowding, sanitary conditions) is the most frequent way of human exposure to get *Leptospira* infection (Fernandes et al. 2019). The global epidemiological status of LPS is fragmentary but in humans an estimated 1.03 million infection cases were reported in 2020 (Calvopiña et al. 2022). It's considered as a major cause of economic loss that can be attributed to the cost of treatment, high culling and low pregnancy in animals (Tomckowiack et al. 2022). The diagnoses are only a key step for a better outcome that depends on the sample availability. Various laboratory tests are used for the detection of the LPS include culturing, molecular, serological and microscopic evaluation (Musso and Scola 2013). LPS can better be treated with antibiotics (penicillin, ampicillin, streptomycin, erythromycin, tetracycline and doxycycline) but prevention can reduce the infection risk by taking sanitary measures (Lucheis and Ferreira 2011).

Clinical Manifestation

LPS may exist in 4 forms including per-acute, acute, sub-acute and chronic form. The clinical manifestations of LPS have non-specific symptoms in humans like fever, headache, myalgia, acute heart failure, hemoptysis and several organ injuries (Costa et al. 2015). Other non-specific symptoms are similar to those seen in dengue, hemorrhagic fever, rickettsial infection, malaria and bacterial sepsis. Approximately 10% cases lead to severe disease (Haake and Levett 2015; Adler and Alejandro 2010). In humans, disease is known by various local names like Fort Bragg fever, Rice field fever, Host Pea Picker's fever, Weill's disease, pretibial fever, Sugar cane cutter fever and Swine herder's fever (Rajapakse 2022; Bhatia and Umapathy 2015).

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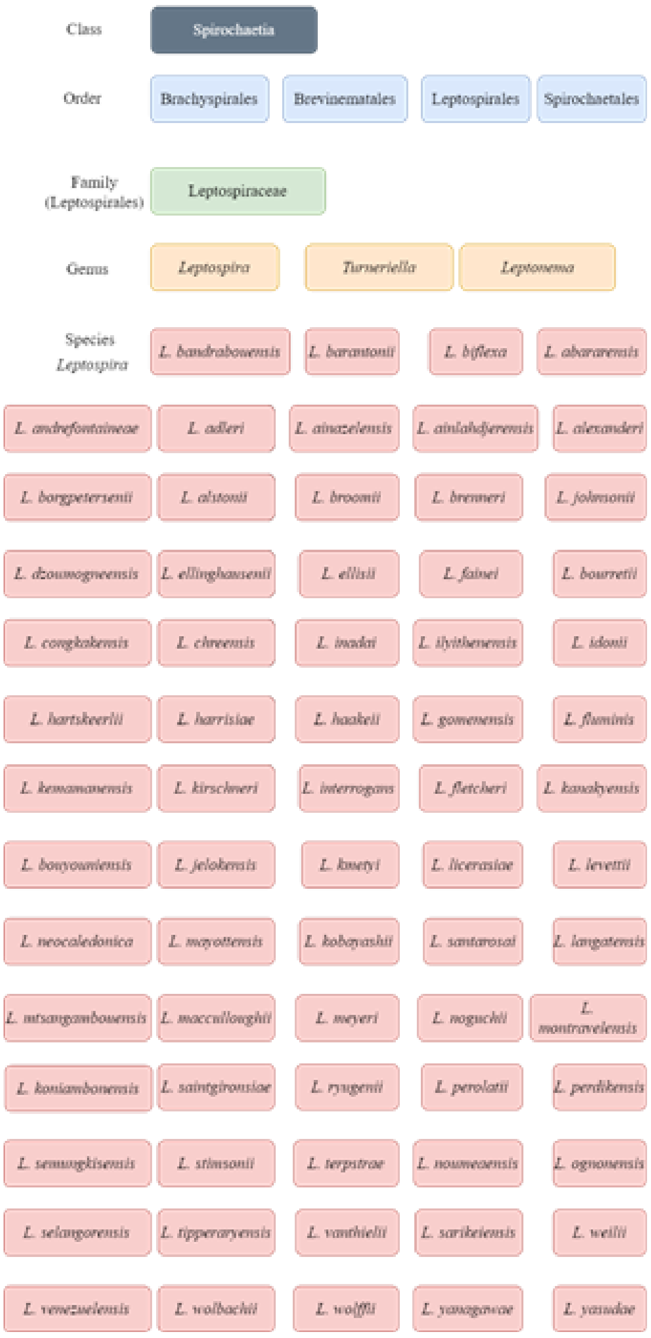

Fig. 1: Taxonomy and Classification of *Leptospira*.

LPS is characterized by a broad range of clinical signs and symptoms in animals with minor differences in affected species. In bovines, it is associated with reproductive problems like stillbirth, abortion, fetus and fetal membrane retention, infertility, low milk yield and calves' death (Correia et al. 2017). Abortion and stillbirth are the most serious clinical events due to pathogenic *Leptospira* infection. Abortion usually occurs during last trimester of pregnancy. Infertility and low milk production occur in pregnant and lactating animals due to the proliferation of *Leptospira* pathogen in the uterus and mammary gland (Rajapakse 2022; Yadeta et al. 2016).

In canines, LPS causes acute infection and has been largely described worldwide. Acute infection associated with serogroups; Icterohaemorrhagiae, Canicole and Grippotyphosa. According to several studies vomiting, lethargy, anorexia, polyuria, weight loss, abdominal pain, diarrhea and dehydration are the most common signs in dogs (Ross 2022; Goldstein 2010). The infection may range from mild febrile illness to life-threatening multisystemic disease which is often characterized by pulmonary hemorrhage and hepatic and renal failure with coagulative disorders (Chacko et al. 2021; Al-Sadi et al. 2015).

Diagnoses

LPS diagnosis is based on history, risk factors and clinical manifestation. Difficult and frequent misdiagnosis of *Leptospira* infection due to similar symptoms of various others diseases makes it the most neglected infectious disease (Chancharoenthana et al. 2022; Tomckowiack et al. 2022). Identification of etiology and confirmation of clinical cases is particularly significant to establish proper preventive measures and appropriate treatment in the herd (Tomckowiack et al. 2022; Martins and Lilenbaum 2017). For the proper diagnosis of LPS laboratory diagnosis is essential (Chancharoenthana et al. 2022).

The diagnosis of LPS is difficult that can be performed by directly identifying spirochetes, their components in body tissues and fluids, and detecting antibodies during clinical phases. Culturing and isolation of pathogens enable for the identification of infectious serovars but it is insensitive and several weeks' incubation is required (Pal, Lema, and Atalel 2022). The majority of LPS patients are diagnosed with serologic tests by using either plasma or serum and serum are most frequently used (Ahmad et al. 2005). Several studies revealed that for the diagnosis of pathogenic *Leptospira* in aborted cattle serum, the sample is collected for antibiotic detection. Laboratory diagnosis includes both direct and indirect identification as shown in Table 1 (Chancharoenthana et al. 2022; Gasem et al. 2020).

LPS diagnosis is difficult because it typically necessitates the pathogen isolation that takes time. LPS diagnostic tests should be accurate, easy to use, cheap, interpret easily, and offer outcomes in a few hours. In addition to a Dark Field Microscopy (DFM), Microscopic agglutination test (MAT) can be a simple procedure with less expensive equipment. LPS-related serovars are identified using MAT. Australis, Autumnalis, Bataviae, Canicola, Ballum, Cynopteri, Grippotyphosa, Sejroe, Hebdomadis, Icterohaemorrhagiae, Panama, Ponoma, Pyrogenes, Tarassovi and nonpathogenic serogroups Semaranga are the few antigens to be used in MAT (Soo et al. 2020; Van De Werve et al. 2013). As the live *Leptospira* are utilized as the material, the method is not standardized. LPS can be biologically diagnosed using ELISA, although the technique has significant drawbacks,

Table 1: Laboratory Diagnostic Tests

Direct Method	Indirect Method
Microscopy	Genus Specific antibody test
Immunohistochemistry	Indirect hemagglutination (IHA)
Immunofluorescence	Enzyme-linked immunosorbent assay (ELISA)
immunoperoxidase	Leptospirosis immunoglobulin (IgM) dipstick
Isolation of Leptospires	Microcapsule agglutination test (MCAT)
Deoxyribonucleic acid (DNA) phase contrast staining	Serovar-specific antibody test
	Microscopic agglutination test (MAT)
Warthin Starry silver stain	
Dark Field Microscopy (DFM)	
Animal Inoculation	
Polymers chain reaction (PCR)	
Loop mediated isothermal amplification (LAMP)	
Next generation sequencing (NGS)	

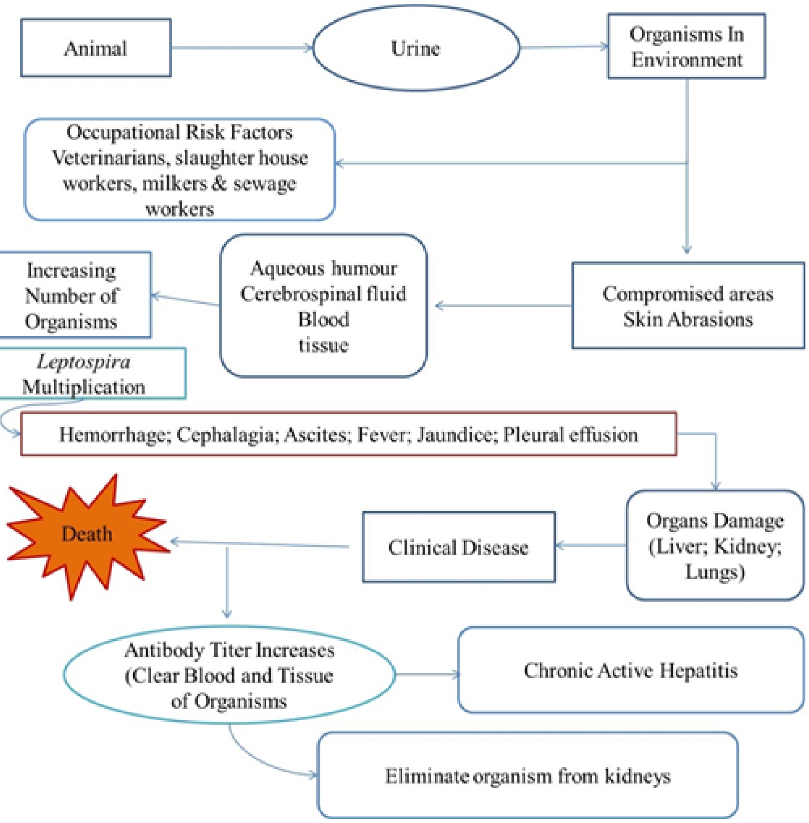
Animal
Urine
Organisms In Environment
Occupational Risk Factors Veterinarians, slaughter house workers, milkers & sewage workers
Increasing Number of Organisms
Aqueous humour Cerebrospinal fluid Blood tissue
Compromised areas Skin Abrasions
Leptospira Multiplication
Hemorrhage; Cephalagia; Ascites; Fever; Jaundice; Pleural effusion
Death
Clinical Disease
Organs Damage (Liver; Kidney; Lungs)
Antibody Titer Increases (Clear Blood and Tissue of Organisms
Chronic Active Hepatitis
Eliminate organism from kidneys

Fig. 2: Transmission and Pathogenesis framework of Leptospirosis (Samrot et al. 2021).

most notably the use of non-pathogen antigen, which makes it possible thatnot all infectious serovars will be detected. By far the most important advantage of PCR is the possibility to confirm the diagnosis of LPS before the appearance of IgM during the early acute phase in infected individuals. Real-time PCR is moreover a quick, sensitive and focused method with lowering the erroneous results from carryover contamination. Cross-reactions caused by the presence of additional diseases could also be found while conducting the ELISA test as shown in Table 2 (Pal et al. 2022; Sykes et al. 2022; Chacko et al. 2021; Budihal and Perwez 2014).

Therapy & Prevention

Treatment of LPS is a big challenge to clear *Leptospira* from tissues. Treatment is usually done with antibiotics and supportive therapies (Ananda et al. 2008). The majority of LPS cases are mild and resolved with antibiotic drugs that are given according to the severity of the illness (Chacko et al. 2021). It's vital to start the treatment to avoid complications until confirmation but the diagnosis must be confirmed (Haake and Levett 2015). In humans, LPS is treated with recommended antibiotics including ampicillin, ceftriaxone and cefotaxime (Panaphut et al. 2003).

Table 2: Laboratory Diagnostic Tests, their targets and Remarks (Sykes et al. 2022; Samrot et al. 2021; Agampodi et al. 2012)

Test	Diagnostic Sample	Target	Remarks
DFM	Urine	*Leptospira*	Low sensitive Require technical expertise
Culture	Blood, Urine	Leptospires	Media Requirement Prolonged incubation Low sensitivity
MAT	Serum	Antibodies against several serovars	Error can occur Live *Leptospira* utilized Suitable for epidemiological purposes High Specificity Can cross validate ELISA finding
ELISA	Serum, Plasma	*Leptospira* IgM, IgG	False Negatives & Positives can occur
DNA (Nucleic acid amplification) test	Blood, Urine, Tissue specimen, CSF	*Leptospira* DNA	Quick Sensitive Low erroneous in result Sensitivity can reduce due to antimicrobial therapy
Histopathology	Kidney tissue specimen	Leptospires	Antimicrobial therapy lead to a false result

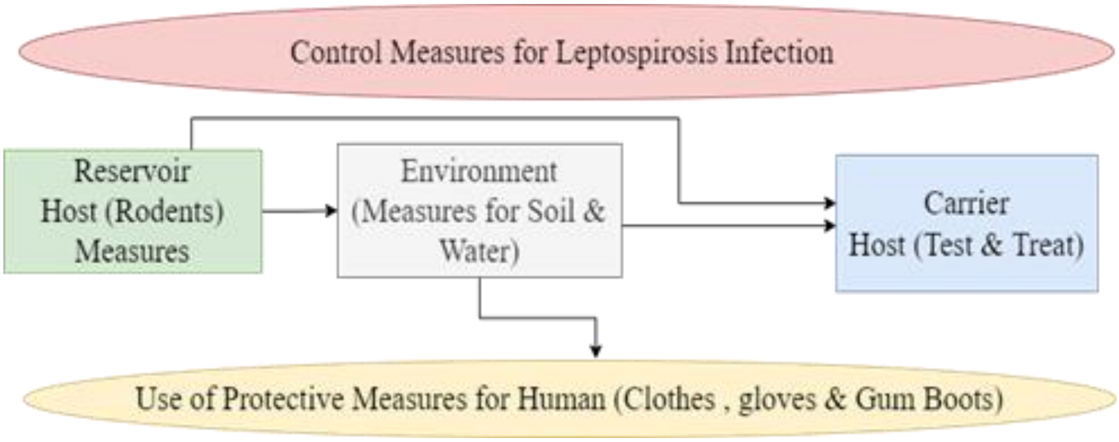
Control Measures for Leptospirosis Infection
Reservoir Host (Rodents) Measures
Environment (Measures for Soil & Water)
Carrier Host (Test & Treat)
Use of Protective Measures for Human (Clothes , gloves & Gum Boots)

Fig. 3: Control Strategies for Leptospirosis (Vijayachari et al. 2008).

Adult patients with early onset of infection can be treated with azithromycin and doxycycline. Antibiotic therapy is effective within 7-10 days but benzylpenicillin is prescribed only for 5 days. Hypersensitive or allergic patients to the penicillin group can be treated with erythromycin for 5 days and doxycycline for 10 days. It is revealed from previous studies that penicillin G is ineffective to use due to the development of resistance. Tetracycline (doxycycline) in young children, pregnant ladies and kidney dysfunction patients is contraindicated (Lawrence 2022; Brett-Major and Coldren 2012).

In animals, various antibiotics are used including ampicillin, ofloxacin, enrofloxacin, ciprofloxacin, doxycycline, and oxytetracycline for cure in hamsters, swine and cattle (Langston and Heuter 2003). Erythromycin and aminoglycoside are effective but aminoglycosides should be avoided in renal dysfunction (Soo et al. 2020; Langston and Heuter 2003).

Fluid therapy, blood transfusion and other supportive care are also vital that depend on the animal and its needs. Administration of fluids is mostly suggested as the first consideration to correct hypotension, hypovolemia and renal failure. Urine output should be assessed after rehydration. If the hydrated patient's urine output remains low, then it should be treated with diuretics (Bilal et al. 2018; Pappas et al. 2008; Langston and Heuter 2003).

Although many therapies are used to control the transmission of LPS infection and reemergence but preventive measures are always better. The preventive measures include spreading awareness regarding transmission route, avoiding contact with animals, soil, water, and avoid travelling in the endemic areas as much as possible is an ideal path to eradicate LPS. The proper use of protective equipment such as gloves, and boots can prevent the contact of farmers, agriculture workers, animal caretakers, athletic events, and flood relief people with the infectious agents as shown in Fig. 3 (Karpagam and Ganesh 2020; Bavia et al. 2019). These people must be well trained and alert to not get exposed and infected (Jorge et al. 2018). Folks should be advised not to swim in contaminated water bodies and avoid exposure and contact with pets and infected animals. Vaccine design is also a major challenge since 1886 for LPS (Karpagam and Ganesh 2020).

Conclusion

Leptospirosis is a zoonotic but treatable disease found worldwide having major prevalence in developing countries. The disease infects both humans and animals. In humans, it is locally known as Weil's disease. The clinical manifestation and severity of the disease are highly variable. The main source of infection is the urine of contaminated animals. Laboratory tests confirm the diagnosis of infection. Available treatment for the zoonotic diseases involves the use of antibiotics. Sanitary measures are the most important control measures in animals and humans.

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Paratuberculosis: A Potential Zoonosis

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INTRODUCTION

The zoonotic significance of paratuberculosis has been debated for a long time (Dalziel 1913). After the identification and isolation of *Mycobacterium avium paratuberculosis* (MAP) in humans suffering from Crohn's disease in 1986 (Chiodini et al. 1986), the attention of many researchers diverted to finding the zoonotic potential of paratuberculosis (Grant 2005). Furthermore, MAP is considered as an important agent in the pathogenesis of certain human auto-immune diseases. These diseases include human immunodeficiency disease, diabetes mellitus, encephalomyelitis disseminate, chronic autoimmune thyroiditis, Besnier-Boeck-Schaumann disease (Sechi et al. 2008; Paccagnini et al. 2009; Sisto et al. 2010; Cossu et al. 2013). The zoonotic potential of paratuberculosis is based on the analogical relationship of clinical symptoms between Johne's disease in animals (i.e. both ruminants and non-ruminants) and Crohn's disease in human beings (El-Zaatari et al. 2001). The pathogenic role of MAP is associated with several other factors i.e., intrinsic and extrinsic that can influence the onset of chronic disease in humans. The intrinsic factors include the inability of one's immune response to withstand the harms of the pathogen due to genetic problems or injury to lymphoid tissues. On the other hand, extrinsic factors include exposure of humans to the causative agent of disease or disturbance in the microbial flora of the gastro-intestine. All these factors combine to the onset of the pathogenesis of paratuberculosis in humans (Chiodini 1989; Naser et al. 2014). Moreover, antigenic molecular mimicry is also hypothesized to influence the generation of the autoimmune response in humans exposed to the MAP (Cossu et al. 2013).

Paratuberculosis—Animal-based Perspective

Paratuberculosis, also known as John's Disease, is a well-known infectious chronic disease characterized by granulomatous inflammation of the intestines (Eslami et al. 2019). The disease is caused by acid-fast bacteria belonging to the genus *Mycobacterium*, specie *avium*, and sub-specie *paratuberculosis* (Malvisi et al. 2016). The causative agent of this disease is an obligate parasite that is dependent on its host cells and necessarily requires host cellular machinery for division and propagation (Arsenault et al. 2014). The etiological agent was first recognized 100 years ago in large domestic ruminants suffering from intestinal inflammation for a long period by two German researchers (Jöhne and Frothingham 1895). Later on, the disease was recognized as Johne's Disease and the associated pathogen was identified throughout Europe in 1906 (McFadyean 1906) and America in 1908 (Pearson 1908). The *Mycobacterium avium paratuberculosis* (MAP) infects a wide range of animal species. The disease is commonly found in the domestic population of ruminants including cattle, sheep, and goats as well as in wildlife populations e.g., deers, foxes and cotton tail rabbits (Rohde and Shulaw 1990; Whittington and Sergeant 2001; Anderson et al. 2007; Münster et al. 2013). Within the animal herd, the disease may be vertically transmitted from the pregnant dam to the fetus during pregnancy (Whittington and Windsor 2009), or during the neonatal life period of calves through ingestion of infected milk/colostrum (Khol et al. 2013) or other contaminated tamaterial e.g., soil, feces, etc (Aly et al. 2012).The body fluids of infected animals including saliva, semen and blood, etc., may also carry the infectious agent and be transmitted to healthy animals upon exposure to these fluids (Abbas et al. 2011; Sorge et al. 2013). Although MAP is majorly transmitted through the vertical mode of transmission, it may also be transmissible horizontally from infected calves to healthy calves, or from infected calves to wildlife reservoirs and vice versa (Sweeney 1994). On exposure of the animal to the MAP through the oral route, the bacterial organism either settles in the ileum directly or becomes stuck up in the lymphoid organ i.e., tonsils. From tonsil crypts, it gains entry into the blood or lymphatic circulation, and ultimately reaches the ileum and mesenteric lymph nodes (Payne and Rankin 1961; Sweeney et al. 2006). The M-cells in the payer patches of the small intestine assist the movement of MAP across the mucosal epithelium of the small intestine. After crossing the

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epithelial barrier, the MAP reaches the submucosa of the intestine and is phagocytized by the sub-mucosal macrophages (Momotani et al. 1988). The persistent presence of MAP in macrophages leads to the onset of granulomatous inflammatory reaction in the intestine and gut associated lymphoid tissues. This leads to the formation of anti-MPA immunoglobulins due to Th2-lymphocytes activation and release of certain cytokines e.g. IL-4 and IL-10 (Stabel 2000). The MAP starts replicating in the macrophages and then spreads to the uterus, salivary glands, muscles, and other body organs. Thus, the clinical symptoms of disease start appearing and pathogen shed in the faeces and in fluids of infected animals (Sweeney 2011; Arsenault et al. 2014). The appearance of clinical signs in MAP infection is associated with the severe economical losses to the farmers of dairy cows due to prolonged course of disease (Khol et al. 2017). The role of MAP in causing disease in animals is well understood, however, the zoonotic potential of this disease in humans is still debatable.

Paratuberculosis—Human-based Perspective

Zoonotic Transmission

MAP is an environmentally stable pathogenic organism that is viable in the soil and water bodies for approximately 4 months (Garvey 2020). After bacterial shedding in feces, the pathogen resides in the soil of a farm or grazing land. The pathogens may contaminate the soil of pasture due to the utilization of animal manure as fertilizer in fodder crops and reside in the roots and shoot system of plants (Grewal et al. 2006; Kaevska et al. 2014). It may also contaminate the water bodies where municipal water is drained off and resides in biofilms (Botsaris et al. 2016). Therefore, the transmission of MAP from animal reservoirs to humans mainly occurs through direct contact of humans with animal feces, contact with contaminated soil, or consumption of contaminated water (Garvey 2018). The consumption of contaminated milk or milk products is also thought to be a potential source of MAP exposure in humans. Different research studies have confirmed the presence of MAP in the milk of MAP-infected dairy animals and some fermented dairy products e.g., yogurt and cheese (Eltholth et al. 2009; Gill et al. 2011; Van Brandt et al. 2011; Galiero et al. 2016). The presence of MAP in the meat of infected animals has also been previously reported (Alonso-Hearn et al. 2009). Besides oral transmission of MAP, different researches have also suggested the aerosol transmission of the MAP from host animals to humans (Eisenberg et al. 2011; Rhodes et al. 2014). Humans are majorly exposed to the pathogen during the so-called "eclipse phase" of the pathogen in the host animals due to ignorance of the farmers for screening of MAP in their animal herds, trade of infected animals, and inefficiency of the MAP diagnostic tests (Naser et al. 2014; McNees et al. 2015; Dow and Alvarez 2022).

The Global Incidence of Different Human Diseases Associated with Paratuberculosis

The zoonotic potential of MAP was the first time evidenced after the isolation and detection of the pathogen in clinically sick patients suffering from gastro-intestinal inflammation and exhibiting clinical signs similar to that of Johne's disease in the animal. This disease is known as infectious bowel disease (IBD) or Crohn's disease and it affects one to two million people around the globe (Molodecky et al. 2012). Later on, MAP was also reported to infect patients suffering from some autoimmune disorders. All these ailments are chronic with no specific treatment and millions of people around the globe suffer from these diseases annually. These diseases include juvenile diabetes, adult-onset diabetes, encephalomyelitis disseminate, chronic auto-immune thyroiditis, and Besnier-BoeckSchaumann disease (Waddell et al. 2015). Juvenile diabetes is a genetic disorder in which the pancreatic cells are unable to produce enough insulin for the regulation of the glucose-glycogen cycle. About 2-5% of people are affected by this type of diabetes in the world annually (Tao et al. 2015). Adult-onset diabetes is a condition seen in adults in which the sugar level of the patient is increased above the normal range due to the inability of the body cells to utilize insulin. Approximately, 90 % of adults in the world suffer from this type of diabetes in the world (Zheng et al. 2018). Encephalomyelitis disseminata is an auto-immune disorder characterized by the injury to the myelin covering of the neurons, thus, creating a hindrance in the transmission of neurological signals to the brain and spinal cord. About 0.3-0.6 individual per one hundred thousand people suffer from this disease in the world each year (Pohl et al. 2016). Chronic auto-immune thyroiditis is an auto-immune inflammatory condition that is characterized by hypothyroidism due to the attack of immune cells on butterfly-shaped thyroid glands in the body. About 3.5-5/1000 females and 0.6-1/1000 males suffer from this condition in the world each year (Ragusa et al. 2019).

Paratuberculosis and Infectious Bowel Disease (IBD)

The term IBD refers to two conditions affecting the gastrointestinal tract in human beings i.e., Crohn's disease and ulcerative colitis. In both of these conditions, the gross and microscopic inflammatory appearance of the intestinal wall is analogous to that observed in animals suffering from paratuberculosis (Gill et al. 2011). The formation of lesions in the intestines results in the appearance of several clinical signs including loose motions, abdominal pain, and malabsorption of nutrients through villi in the small intestine. Moreover, the condition becomes more severe and chronic after the involvement of the immune system response (Thia et al. 2010). The selective absorptive capability of the intestine is compromised and the causative agent of paratuberculosis (i.e. MAP) gains access to the different body organs through the circulatory system (Naser et al. 2004). Moreover, similar to the shedding of bacteria from the mammary glands of animals, studies have shown the shedding of MAP in the milk samples from the breast of patients suffering from Crohn's disease (Naser et al. 2000; Bannantine et al. 2014). Another similarity between paratuberculosis and IBD is the mutation of bacterial sensing immunogenic gene i.e., NOD2. The mutation of this gene leads to the defective or no production of NOD2-protein associated with the immune function of certain immune cells e.g. macrophages and paneth cells in the intestinal epithelium (Kuenstner et al. 2015). This genetic relation between Johne's and Crohn's disease suggests that both diseases share a common etiological agent. The MAP is observed in the histopathology of intestinal tissue biopsy samples under 100x objective magnification using acid-fast staining (Jeyanathan et al. 2007). Moreover, the PCR of MAP-specific genes has shown a positive presence in the biopsy samples from intestine (Bull et al. 2003). The doubts regarding etiological resemblance of Johne's disease and Crohn's disease vanished when the successful isolation and culturing of MAP from humans caused active infection in intestine of MAP inoculated goats (Van Kruiningen et al. 1986). All these studies indicate a strong zoonotic relationship between Johne's and Crohn's disease.

Paratuberculosis and Diabetes Mellitus

It is a disease that is characterized by the inability of endocrine cells of the pancreas to produce insulin due to the autoimmunity of T lymphocytes against proteins (heat shock protein and insulinoma-linked proteins) and an enzyme (glutamic acid decarboxylase) in the cells of islets of Langerhans (Kerner and Brückel 2014). Paratuberculosis and insulin-dependent diabetes (also known as diabetes mellitus type-I) are reported to share some genetic similarities (Rani et al. 2010). The mutation of the SLC11A1 gene leads to the protection of MAP from the host's immunity. This gene encodes Natural Resistance Associated Macrophage Protein-1 (MRAMP1) which is a divalent metal ion transporter membrane protein of phagocytic cells and acts by resisting the host cells against several types of microbial pathogens (Hsu et al. 2006). The mutation in the gene of this protein is associated with dysregulation of this protein and the inability of the macrophages to destroy the pathogen (Paccagnini et al. 2009). Researchers have successfully isolated the DNA material of MAP from a patient suffering from insulin-dependent diabetes. This DNA has also been reported to be isolated from patients suffering from adult-onset diabetes (also known as diabetes mellitus type-II). However, the prevalence of MAP is much less (8%) than reported in the case of insulin-dependent diabetes i.e., 63% (Rosu et al. 2009; Manca Bitti et al. 2012). The induction of autoimmunity in insulin-dependent diabetic patients is related to the similarity between the epitopes of glutamine decarboxylase enzyme (GAD65) of humans and heat shock proteins (HP65) of MAP pathogen. The GAD65 is involved in the conversion of gutamine to butyric acid and carbon dioxide, whereas, heat shock proteins are produced in response to the environmental stress. The exposure of MAP to humans and expression of heat shock proteins results in the failure of the immune system to differentiate between self and non-self-antigens i.e., GAD65 and HSP65 respectively. Thus, resulting in the induction of an autoimmune response against these two homologous proteins and leading to the injury of islets of Langerhans in the pancreas. The damaged pancreas results in the inability of endocrine cells to produce insulin and ultimately patient suffers from insulin-dependent diabetes (Dow and Sechi 2011; Dow 2012). Moreover, the research studies have also reported similarities between the other membrane protein (zinc transporter 8) in the beta cells of the pancreas and a MAP protein (i.e. MAP 3865c). The reorganization of antibodies against zinc transporter 8 protein and MAP protein is further evidenced, supporting the role of MAP in the production of an autoimmune response in insulin-dependent diabetes (Masala et al. 2014).

Paratuberculosis and Rheumatoid Arthritis

Rheumatoid arthritis is defined as a chronic autoimmune disease characterized by the painful swelling of mobile joints due to immune-mediated inflammatory reactions (McInnes and Schett 2011). The role of MAP in the pathogenesis of rheumatoid arthritis is similar to that of diabetes mellitus. The research studies have indicated the polymorphic role of the SLC11A1 gene in the dysfunctioning of NRAMP-1 proteins and the production of T-lymphocytes against the body's cells, thus, enabling MAP to manipulate and worsen the inflammatory reaction in joints (Yang et al. 2000).

Paratuberculosis and Chronic Autoimmune Thyroiditis

Chronic autoimmune thyroiditis (also known as Hashimoto's thyroiditis) is an immune system-mediated inflammation of thyroid glands resulting in the decreased production of thyroxin (hypothyroidism). The role of MAP in the pathogenesis of this condition follows the hypothesis of molecular mimicry where the failure of the immune system to recognize the self-body cells leads to the production of antibodies against the ZnT8 membrane proteins in the follicular cells of the thyroid gland (Sisto et al. 2010; Niegowska et al. 2015).

Paratuberculosis and Encephalomyelitis Disseminata

Encephalomyelitis disseminata (also known as multiple sclerosis) is an autoimmune disease affecting the nervous tissues of the brain and spinal cord through injury to the myelin sheaths caused by helper T lymphocytes (Cossu et al. 2017). The myelin sheath acts as a protective shield for neurons and is important in the transmission of nerve impulses from the effector organ toward the CNS (Balice-Gordon et al. 1998). Numerous studies reported the high incidence of encephalomyelitis disseminata in Italy (42%) and identification of MAP from affected patients was associated with the environmental exposure of Italians to the pathogen (Cossu et al. 2011; Cossu et al. 2013). However, some researchers have associated this condition with autoimmunity and related genetic predisposing factors that are further strengthened after the exposure of humans to MAP. The formation of myelin shields on neurons is an important phenomenon and any abnormality or disruption in this procedure causes the onset of neurological symptoms in the affected individual (Stiles and Jernigan 2010). The role of MAP in association with this neuro-behavioral condition is attributed to the reactivity of myelin basic proteins with molecularly similar antigenic proteins of MAP and thus, depleting the myelin from the axons of neurons and resulting in disruption of the transmission of a nervous signal across the CNS (Mameli et al. 2014).

Conclusion

MAP in humans plays an important role in the pathogenesis of Crohn’s disease and some autoimmune diseases through the mutation in the genes of certain immune cell membrane proteins, cross-reactivity with cellular proteins, and exhibition of antigenic molecular mimicry. The shedding of bacteria occurs in feces, milk, blood, saliva, semen, etc, and direct contact with these fluids is the very possible root of transmission of pathogens to other animals and human beings. Moreover, the in-utero transmission from infected dams to the fetus in animals, and the consumption of infected meat by humans are also possible routes of transmission of MAP.

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8

Genes for Multidrug Resistance in *E. coli*: One Health Challenge

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INTRODUCTION

Antimicrobial resistance (AMR) has recently been identified as one of the major public health issue affecting millions of peoples' health worldwide due to the quick evolution and spreading of resistant pathogens among humans, animals, and the environment. These antimicrobial resistant pathogens not only create hiddenness in clinician efficiency but also limits the therapeutic options which ultimately leads to increase the cost of health services by increasing infections persistence, rates of morbidity and mortality (Prestinaci et al. 2015; Morehead et al. 2018). At present it is estimated that total deaths related to AMR accounts for 700,000 annually and it is predicted to be 10 million deaths/ year by 2050. Antimicrobial resistance is becoming a complex and multifaceted threat not only for humans but also for animals and environment due to transmission of antibiotic resistance genes through movement of bacteria in different ecologies or mobile genetic elements and their acquisition by highly pathogenic bacteria in each interrelated segment (de Kraker et al. 2016).

Commensal *E. coli* usually inhabits gastrointestinal tracts of warm-blooded animals including human as part of their normal flora and is also ubiquitous in the environment. Commensal *E. coli* generally do not cause any infection to the host but harboring transmissible resistance elements have led to the evolution of this bacteria into extremely modified antibiotic-resistant pathogen and now it's becoming a challenge as infection caused by resistant patho-types are difficult to manage (Rajagopal et al. 2021). The emergence of antibiotic resistant is not restricted to *E. coli* strains isolated form human but also reported in animal and environment origin strains. The emergence of AMR *E. coli* is mainly observed due to overuse of antibiotics in veterinary and agriculture settings (Shakya et al. 2016; Amézquita-López et al. 2019).

MDR Emergence: Global Drug Resistance Pattern Reported Data from Animal, Human and Environment

Antimicrobials are routinely used to treat diarrhea, gastroenteritis, mastitis and urinary tract infection caused by *E. coli* in animals. However, use of antimicrobials for treatment and as a growth promoter have been associated to the development of multidrug-resistant (MDR) *E. coli* that is not only becoming a public health threat but also poses economic burden for farmers due to increase in treatment cost. These AMR bacteria and/or pathogens can be transmitted from animals to human and then spread to their environment and enter in food chain. Many studies showed the spread of antimicrobial resistant *E. coli* among chickens, humans, livestock, and respective environmental settings (Heuer et al. 2011; Novais et al. 2013; Wichmann et al. 2014; Aworh et al. 2016; Amézquita-López et al. 2019). Different studies reported that *E. coli* isolated from livestock demonstrated resistance against different classes of antimicrobials (Penicillin, macrolide, aminoglycoside and sulfonamide) (Brennan et al. 2016; Astorga et al. 2019; Azabo et al. 2022). Especially, food producing animals are becoming a potential source of dissemination of antimicrobial resistant pathogen into environment through their feces as poultry waste is routinely used for the feed of fish and shellfish (Nhung et al. 2016). Globally, different antibiotics are used in production and treatment of disease in Cattles and Buffaloes and are becoming an important reservoirs of AMR bacteria. In 2021 a study was conducted in Northeast Mexico and isolates from cattle feces and carcasses showed 72.7% overall prevalence of MDR *E. coli* (Martínez-Vázquez et al. 2021). Some other studies have also described the incidence of MDR *E. coli* as 44 %, 69%, 56% and 37% in Egypt, Portugal, France and Jordan respectively (Obaidat et al. 2018; Amador et al. 2019; Bourély et al. 2019; Ramadan et al. 2020).

A study was conducted in China and reported that extended spectrum β-lactamase (ESBL) *E. coli* was found in the soil that was treated with pig dung and genetic analysis of isolates from soil have showed above 90% similarity with pig dung isolates (Gao et al. 2015). Another study reported the isolation of genetically related *E. coli* from pond water and duck fecal samples (Ma J et al. 2012). Such studies indicating

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Table 1: Antimicrobial Resistance Profiling of MDR *E. coli*.

Country	Strain	Resistance	Reference
Dubai, UAE	*E. coli*	Ampicillin, Tetracycline, Co-trimoxazole, Gentamicin and Enrofloxacin	Abdelwahab et al. 2022
Austria	*E. coli*	Oxacillin, Ceftazidime, Colistin, Nalidix acid, Sulfamethoxazole, Tylosin	Hess et al. 2022
Romania	*E. coli*	Amplicillin, Fosfomycin, Norfloxacin, Amoxicillin/Clavulanic Acid, Gentamicin	Imre et al. 2022
Ghana	*E. coli*	Ceftazidime, Cefuroxime, Augmentin	Kichana et al. 2022
South Korea	*E. coli*	Tetracyline, Ampicillin, Ciprofloxacin, Streptomycin, Chloramphenicol	Song et al. 2022
Germany	*E. coli*	Streptomycin, Ampicillin, Amoxicillin/Clavulanic acid, Sulfonamides, tetracycline	Gross et al. 2022
Bangladesh	*E. coli*	Levofloxacin, Ciprofloxacin, Cefotaxime, Imipenem, Doxycycline	Mandal et al. 2022
Norway	*E. coli*	Ceftriaxone, Ceftazidime, Cefotaxime, Cotrimoxazole, Ampicillin, Ciprofloxacin, Meropenem, Imipenem	Pulingam et al. 2021

a strong relation of AMR *E. coli* transmission from animals to environment. Recently a study conducted in 2022 on sheltered dogs in Kanto region of Japan and detected MDR *E. coli* in 18 samples by detecting β-lactamase genes (*blaTEM*, *blaDHA-1*, *blaCTX-M-9* group and *CTX-M-14*) and quinolone-resistance protein genes (*qnrB* and *qnrS*) (Hata et al. 2022). Previous studies have reported that MDR bacteria in the cohort animals can be a health risk to humans (Shakya et al. 2016; Rajagopal et al. 2021).

Epidemiology and Global Burden of MDR *E. coli*

Antimicrobial resistance in bacteria is one of the major public health concerns that may lead to the deaths of 10 million people annually by the end of 2050. Therefore, World Health Organization (WHO) and some other regulatory bodies have declared AMR as the most emergency condition that should be immediately addressed (Murray et al. 2022). AMR resulted in the deaths of 700,000 individuals in the year 2014 and the estimated number of deaths in different regions of the world by 2050 are given in Fig. 1. If this issue left unnoticed, circumstances may lead to the widespread emergence of extremely pathogenic and multi-drug resistant bacteria that never existed before (Pulingam et al. 2021).

According to the data retrieved from WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) report 2021, it became evident that *E. coli* and *K. pneumoniae* are the most antimicrobial resistant pathogens isolated from community, hospital, and unknown origins. MDR *E. coli* strains isolated from different regions of the world displayed resistance against many of the commonly used antibiotics and even against 3rd generation cephalosporins as summarized in Table 1.

Resistance of *E. coli* against 3rd generation cephalosporins was at maximum prevalence level of 58.3% in economically developing countries while that of 17.5% for economically developed countries (Pulingam et al. 2021). Colistin is the last resort antibiotic used against MDR *E. coli* that are even resistant against carbapenems but many different strains of *E. coli* have also developed resistance against colistin through mutations in the mobile colistin resistance (*mcr*) genes. Ten different homologues of *mcr* have been known to cause colistin resistance in MDR *E. coli* and their prevalence in different continents of the world is clearly depicted in Fig. 2 (Dadashi et al. 2021).

Low- and middle-income countries have very high prevalence of diseases involving MDR *E. coli* but due to their inadequate surveillance systems, complete data is not available (Peirano et al. 2022).

Genetic Variability of *E. coli*

Genetic variability and diversity of *E. coli* is mainly due to genomic plasticity, a large gene pool, worldwide dissemination and ubiquity that plays an important role in making *E. coli* a priority pathogen resulting in extremely high drug resistance and virulence (Horesh et al. 2021). Majority of the *E. coli* are nonpathogenic; however, their genetic clusters are highly variable in host and country to country (Leekitcharoenphon et al. 2021). Horizontal Gene Transfer (HGT) is the major process responsible for genetic variability and it has been estimated that base composition and phylogenetic analysis of *E. coli* genomes showed 24.5% genomes were acquired through HGT (Yu et al. 2021). HGT involves the exchange of genetic material between different species through conjugation, transformation, or transduction. In addition to this, mutational variability is also one of the major factors of genomic diversity in *E. coli* (Pulingam et al. 2021). This higher genomic diversity makes *E. coli* more host and niche specific and diverse at the same time. *E. coli* phylogenetic relationship demonstrates its diversity that exhibits a wide host range including human, wild and domestic animals. *E. coli* has been reported in infections emerging from different environments and around the world with some common and diverse genes clusters (Fig. 3). Over the last 100 years *E. coli* host shift also taken place jumping from wild animals to domestic animals in Mexico. Such host shift pattern might be available throughout the world, as around the world anthropogenic activities and industrialization disturbed the natural habitat of the wild animals which resulted in such shifts (Fig. 4) (Yu et al. 2021).

Genes for Multi-drug Resistance in *E. coli*

Genes encoding antimicrobial resistance against different antibiotics classes have emerged in *E. coli* from both humans and animals' origins. *E. coli* exhibits extremely great genetic diversity and, antimicrobial resistance (AMR) genes in *E. coli* are located variably on chromosome and plasmids.

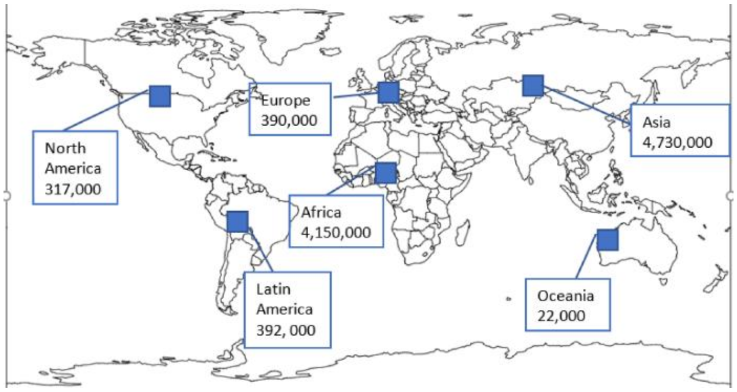

Fig. 1: Estimated number of deaths in diverse areas of the world by 2050 (Pulingam et al. 2021).

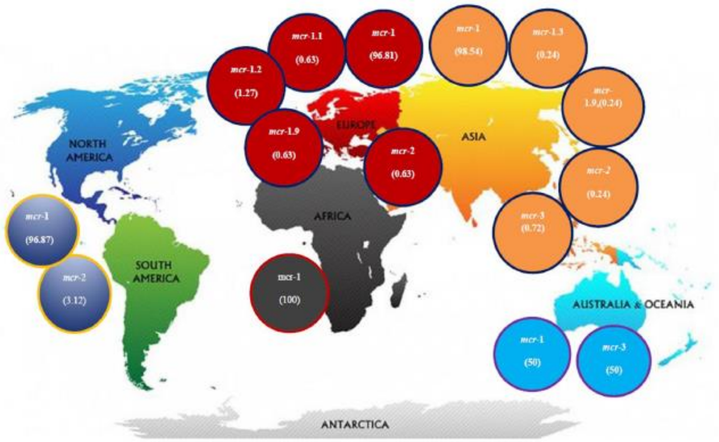

Fig. 2: Dissemination of colistin resistant *E. coli* strains having *mcr* homologues (Dadashi et al. 2021).

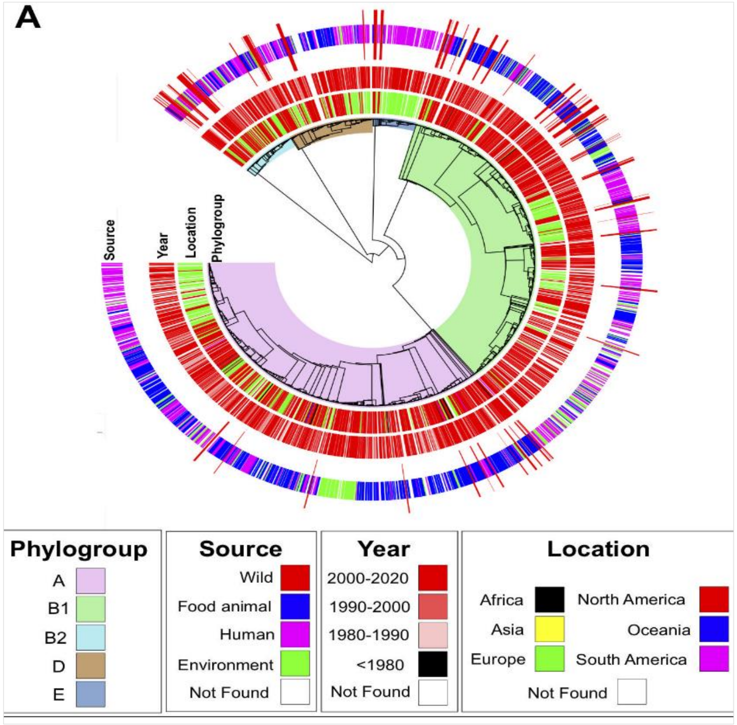

Fig. 3: Phylogenetic map of *E. coli* genomes isolated from different hosts, locations, and years (Murphy et al. 2021).

It also contains a wider variety of mobilome. Most of the AMR genes in *E. coli* are moved through different mobile genetic elements (MGEs). Intracellular transposition make these genes mobile via plasmids and transposons. In other case integrative MGEs play role to move these genes. Some genes are also the part of integrons and move by insertion sequences.

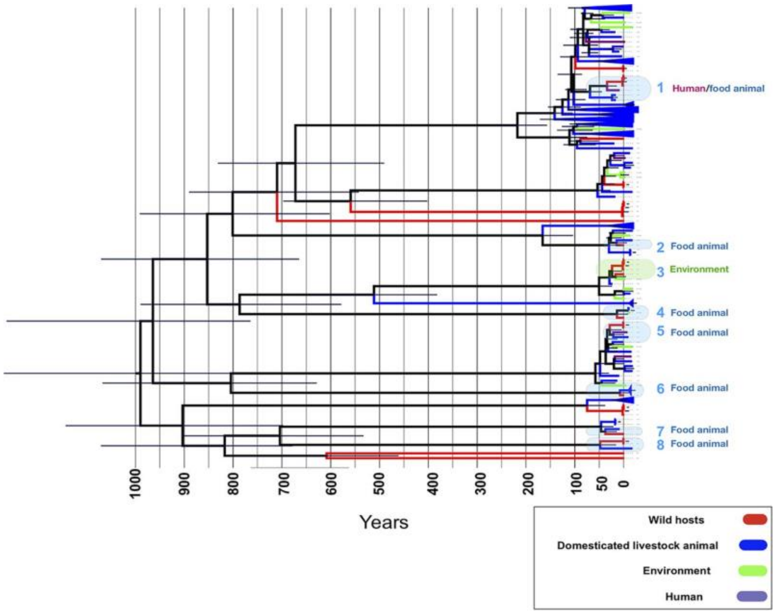

Fig. 4: Phylogenetic analysis showing mixing of wild and nonwild hosts from last 100 years, which shows the *E. coli* jumping events among wild, domestic animals, human and environment (Murphy et al. 2021).

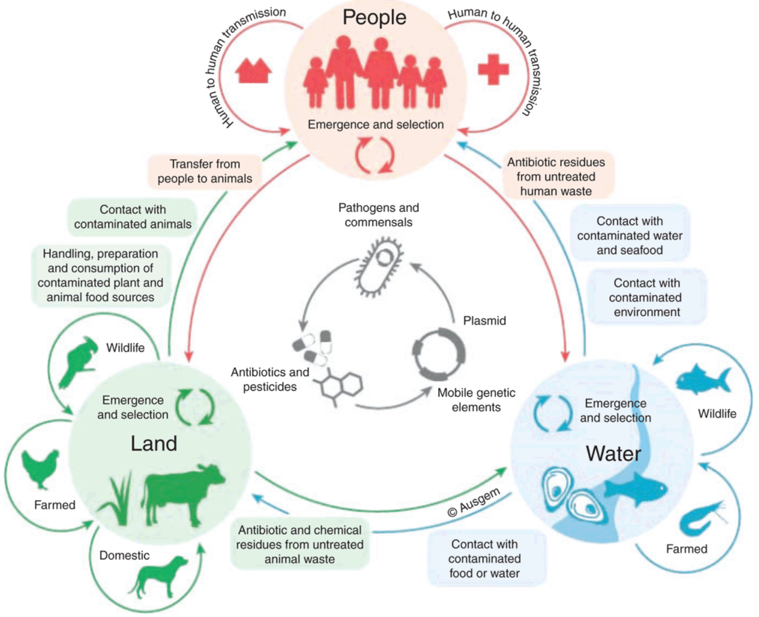
People
Human to human transmission
Human to human transmission
Emergence and selection
Transfer from people to animals
Antibiotic residues from untreated human waste
Contact with contaminated animals
Pathogens and commensals
Contact with contaminated water and seafood
Handling, preparation and consumption of contaminated plant and animal food sources
Contact with contaminated environment
Plasmid
Wildlife
Antibiotics and pesticides
Mobile genetic elements
Emergence and selection
Land
Emergence and selection
Water
Wildlife
Farmed
Domestic
Antibiotic and chemical residues from untreated animal waste
© Ausgem
Contact with contaminated food or water
Farmed

Fig. 5: Illustration of AMR genes transfer to Human, Animal and Environment posing severe concern for One Heath (Djordjevic and Morgan 2019).

AMR genes and diverse MGEs in *E. coli* are transferred via plasmids, thus making horizontal gene transfer (HGT) a major component in AMR genes spread. Drug resistance in *E. coli* is mainly caused by mutational modifications as well as by gene transfer methods including conjugation, transformation and transduction. Spontaneous mutations account for resistance in *E. coli* against Rifampicin by altering the *rpoB* gene (Leekitcharoenphon et al. 2021, Pulingam et al. 2021). Due to the extraordinary diversity of the genome and wider host range of *E. coli*, finding new AMR genes and their spread mechanism is still under research, however a number of AMR genes around the world has been identified in vast variety of host ranges for carrying antimicrobial resistance.

AMR Genes Mobility and One Heath Challenge

Higher diversity and variability of MGEs in *E. coli* presumably makes AMR genes more vulnerable to transfer not only within species but to intraspecies normal flora, pathogens and environmental microflora and then becomes a constant challenge for human animals and environment. Multiple AMR genes harboring MDR and XDR strains have been isolated throughout the world from human, livestock, wild animals, soil, water and food. This greatly posing a concern that along with human, animal and environmental health, food security will also be a one of the major changes due to the spread of AMR in future (Djordjevic and Morgan 2019). Fig. 5 shows an illustration of AMR genes transfer through HGT and impact on One Heath.

Conclusion

E. coli is gram negative bacteria, having wide variety of host range, ubiquitous in environment due to flexible growth requirements and highly diverse genome. The genome of the *E. coli* harbors several AMR genes which are located on chromosome and plasmids. The presence of wide variety of mobile genetic elements makes AMR genes more vulnerable to move intracellularly and extracellularly. Due to the widespread of AMR genes globally MDR and XDR strains of *E. coli* has been emerged. Antimicrobial resistance is a rapidly expanding issue in the world. On every passing day AMR genes are being spread at an alarming rate. This spread of genes is among interspecies as well intraspecies through horizontal genes transfer and this spread posing a severe heath concern for human, animal and environment.

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9

The Anti-*Echinococcus* Potential of Botanicals and Nano Green Particles: Recent Updating

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INTRODUCTION

Echinococcosis is a zoonotic parasitic disease with global distribution. It is endemic in rural sheep-raising areas. In Iraq, hydatid cysts disease is hyperendemic, with high socio-economic effects due to the infection of humans and their livestock (Benyan et al. 2013; Shnawa et al. 2021). The parasite has an indirect life cycle requiring two hosts. First is the intermediate host, in which the cysts can develop in several organs of humans and domesticated mammals such as sheep, cows, buffalo, goats, camels, and pigs. Second is the final host in which the adult worms develop in the small intestine of carnivorous animals such as dogs, and other wild canids, which pollute the soil with the infective eggs evacuated with their feces (Issa et al. 2022).

Echinococcus granulosus is a parasitic worm (Cestoda) and is the causative agent of hydatidosis or hydatid cysts disease. This is attributable to the development of the larval stage (hydatid cyst) in various human organs (Hizem et al. 2020). The main way that humans become infected is by consuming ova of the parasite that are secreted with the faeces of the definitive host and contaminated food, soil, and water. The ingested eggs hatched and released embryos in the small intestine of the intermediate hosts, which then passed through the intestinal wall and wander through the bloodstream to various tissues, primarily the hepatic and pulmonary tissues, where they developed to give the larval stage or the hydatid cyst (Eckert et al. 2017).

Currently, treating hydatid cysts appears to be difficult and complex; however, there are a number of therapeutic approaches available, including the use of chemical agents like benzimidazole derivatives, PAIR (denoting puncture, Aspiration, Instillation, and PAIR (denoting puncture, Aspiration, Instillation, and Re-aspiration), surgery and even "watch and wait." (Craig et al. 2007). The most significant problem after hydatid cyst surgery is protoscoleces dissemination into the patient's viscera as a result of cyst rupture and hydatid fluid leakage, ultimately resulting in serious complications (Ormeci 2014). Meanwhile, studies revealed that the common scolicidals had some side effects, including cirrhosis, biliary cholangitis, and sclerosing cholangitis (Rajabi 2009; Sharafi et al. 2017). According to the literature, PAIR reportedly uses a variety of scolicidal agents, including albendazole, 95% ethanol, hypertonic saline solution, H_2O_2, silver nitrate, and cetrimide. However, many of these agents have side effects that limit how often they can be used to treat hydatid cyst disease (Sungur 1979). These factors motivate scientists to develop a novel scolicidal medication to treat Cystic Echinococcosis (CE). For CE to be successfully treated, research into safe and efficient scolicidal agents for surgery is urgently needed.

Due to their ease of use, eco-friendliness, accessibility, and nontoxicity, metal oxide nanoparticle fabrication has received a lot of attention over the past few years. In addition to, their significant and distinctive properties, such as their surface-to-mass ratio that is much higher than that of other particles, their quantum properties, and their capacity to adsorb and carry other materials like drugs, probes, and proteins, nanoparticles have become widely used in medical applications (Barzinjy et al. 2020). The success of numerous disciplines and sciences depends on nanotechnology and nanoscience. The materials' nanoscale size makes them suitable for a variety of potential applications, including drug delivery (Anoop et al. 2020), cancer treatment (Tian et al. 2019), medical treatment (Devi et al. 2019) and other applications. Overall, utilizing medicinal plant extracts as potential reducing and stabilizing agents for the synthesis of nanoparticles, such as ZnO nanoparticles, offers several benefits over traditional physical and extremely toxic chemical techniques (Mittal et al. 2013).

Recently, the treatment of hydatid cysts has been motivated to utilize green biosynthesized nanoparticles as innovative scolicidal materials such as Silver, Nickle oxide, Zinc oxide, Titanium oxide, and others, within in vitro, ex vivo, and in vivo studies (Ibrahim 2020; Shnawa et al. 2021).

In previous work, the application of some nanoparticles, particularly the greener fabrication of nanoparticles, for instance, selenium, zinc oxide, silver, gold, and other nanoparticles, as a novel scolicidal alternative to cure echinococcosis had been reviewed (Shnawa 2018; Shnawa et al. 2021).

Albalawi et al. (2020) concluded in a review that the most functional nanoparticles for hydatid cyst disease therapy were metal- NPs, metal oxide-NPs, and polymeric NPs.

Citation: Jalil PJ and Shnawa BH, 2023. The anti-*echinococcus* potential of botanicals and nano green particles: recent updating. In: Abbas RZ, Saeed NM, Younus M, Aguilar-Marcelino L and Khan A (eds), One Health Triad, Unique Scientific Publishers, Faisalabad, Pakistan, Vol. 2, pp: 59-65. https://doi.org/10.47278/book.oht/2023.42

Moreover, Chitosan- NPs were proven to be an efficient and safe scolicidal agent at a minimal dose and within short exposure periods (Firouzeh et al. 2021).

Particles with a single dimension between 1 and 100 nm are known as nanoparticles. NPs exhibit a range of traits depending on their size and surface functionalities. Due to their small size and large surface area, NPs are widely used in a variety of industries, including cosmetics, electronics, and diagnostic and medicinal applications. The mode of entry, cellular uptake, and general toxicity of NPs are all influenced by their size. It has been demonstrated that this size-dependent toxicity also occurs in the liver. Silver particles with a size of 10 nm had a higher tissue distribution and more toxic effects on the liver when compared to larger NPs (40 and 100 nm) (Najahi-Missaoui et al. 2020).

Recent Studies of Greenish Fabricated Zinc Oxide Nanoparticles (ZnO NPs)

In a study, Shnawa et al. (2021) reported that the green synthesis of ZnO NPs originating from *Mentha longifolia L.* leaves extracts has an effective scolicidal possibility. At 400 ppm concentration after 150 min of exposure, the ZnO NP showed the highest scolicidal activity with a 100% mortality rate. The treated protoscolices first displayed loss of viability in the parasite's tegument. This was followed by many morphological changes, such as disorganization of the rostellar hooks and loss of calcareous corpuscles as shown in Fig. 1.

Most recently, in a study, *Lavandula angustifolia* extract was used by microwave technique. Several concentrations of the green synthesized ZnNPs (50, 100, and 200 µg/ml) were used alone and in addition to Albendazole (100 µg/ml) in an *in vitro* and *ex vivo* model. By measuring the Caspase-3 activity of protoscoleces treated with a variety of concentrations of ZnNPs, the induction of apoptosis in hydatid cyst protoscoleces was evaluated. At a concentration of 200 g/ml, ZnNPs had the highest level of scolicidal activity, killing 81.6% of protoscolices. The protoscolices were killed by these nanoparticles and ALZ after 10 minutes of exposure, particularly at a concentration of 200 g/ml (Shakibaie et al. 2022). According to these results, ZnNPs and albendazole together demonstrated a powerful protoscolicidal in vitro and ex vivo nanoparticle model against the treatment of hydatid cysts.

Moreover, *Z. spina-christi* leaf extract was used in another investigation (Shnawa et al. 2022a). After 60 minutes of exposure, 400 g/ml of ZnO-NPs exhibited the highest antiparasitic activity with a 100% mortality of the treated protoscoleces. Many morphological alterations and disorganization of rostellar hooks, presence of tegumental and suckers alteration have also been reported as shown in Fig. 2. These authors documented several morphological alterations. The safety and biocompatibility of this nanoparticle against RBCs were also confirmed in which different dosages of ZnO-NPs were considerably less than the positive control. This research proposes that phytochemicals such as polyphenols in the plant extract behave as reducing and stabilizing agents for the creation of ZnO-NPs (Shnawa et al. 2022a).

The antimicrobial mechanisms of ZnNPs were proposed. These nanoparticles can apply their antimicrobial properties through a variety of molecular mechanisms, including preventing cell growth, changing the permeability of cell membranes, promoting induced programmed cell death (apoptosis), inhibiting the production of H2O2 and thereby inducing oxidative stress, and raising the concentration of Zn in the environment prior to entering the body (Sirelkhatim et al. 2015). Additionally, among the fundamental molecular action processes, there is the motivation of programmed cell death, also known as apoptosis. Recent findings demonstrated that ZnNP doses of 50, 100, and 200 g/ml had induced apoptosis at 13.4%, 27.3%, and 34.8%, respectively, dependent on the caspase-3 enzyme (Shakibaie et al. 2022). Similar to this, earlier studies found that ZnNPs significantly increased apoptosis by upregulating the genes for caspase-3, caspase-8, caspase-9, Bax, LC3-II, Atg 5, p53, and Beclin 1 genes, increasing the production of reactive oxygen species, and decreasing the mitochondrial membrane potential in a variety of cells, including human ovarian cancer cells (SKOV3), and mouse Leydig cell line (Wang et al. 2015; Jiang et al. 2018; Jalil et al. 2022).

Recent Studies of Green-based Synthesized Silver Nanoparticles (Ag NPs)

Ag NP biosynthesis is a very efficient process for creating less toxic nanoparticles. A study utilized three different plant extracts from the leaves of *Piper nigrum*, *Ziziphus spina-christi*, and *Eucalyptus globulus* for the biosynthesis of Ag NPs. The scolicidal activity of these nanoparticles against *E. granulosus* has been studied. The outcomes showed that the scolicidal rate of 47.8% after 45 min for the Ag NPs derived from *Eucalyptus globulus* was comparable to that of albendazole treatment (Salih et al. 2020). The proportion of metallic ions in the solution to the capping and stabilizing agents determines the metallic nanoparticle size. The amount of Ag nanoparticles increased with the amount of capping and stabilizing agents present in the higher concentration of plant extract (Kohan Baghkheirati et al. 2016; Moazeni et al. 2019). The released Ag+ gave rise to the Ag nanoparticles' cytotoxicity. Numerous mechanisms explain cell death through interactions with Ag+. Ag ions can interact with the proteins' disulfide bonds and alter the microorganism's three-dimensional structure. The organisms' functionality is prevented by that interaction (Ahmed et al. 2016). Creating complexes with DNA and RNA, breaking mitochondrial membranes, and proton motive force are some additional mechanisms (Medici et al. 2019).

In a recent study, *in vitro* scolicidal effectiveness of the biosynthesized AgNPs from *Zizyphus spina- christi* leaves against *E. granulosus* protoscoleces was pointed out. Numerous morphologic changes were noted on the protoscoleces by both optical as well as scanning electron

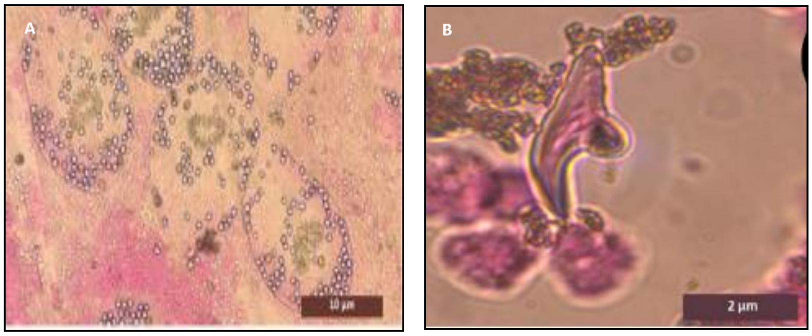

Fig. 1: A&B, dead protoscolices after exposure to ZnO NPs and stained with eosin. The protoscolices are damaged and lose their hooks (Shnawa et al. 2021).

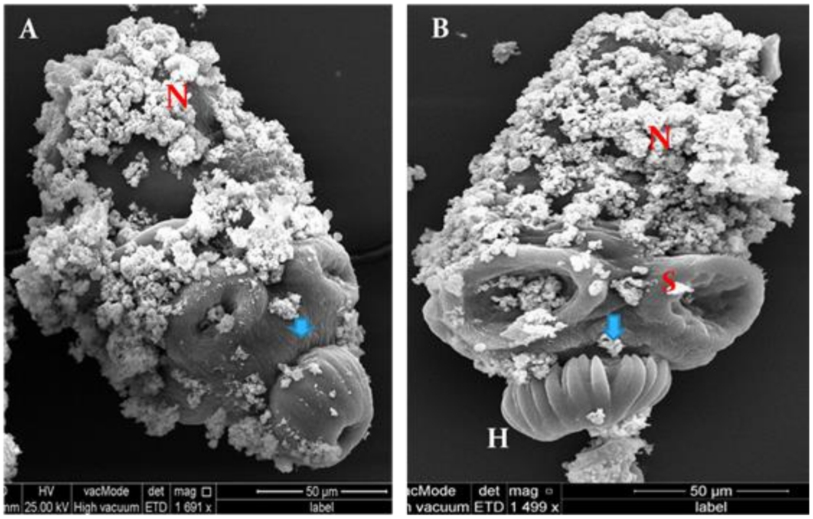

Fig. 2: (A) Evaginated protoscoleces treated with ZnO-NPs. White-colored nanoparticles (N) attached to the tegument of the parasite; the arrow signifies the soma region. (B) Protoscolex incubated with ZnO-NPs, displays contraction of soma region (arrow) And disorganization of rostellar hooks (H), also, the existence of tegumental and suckers alteration; Suckers (S), Hooks (H), (Shnawa et al. 2022 a).

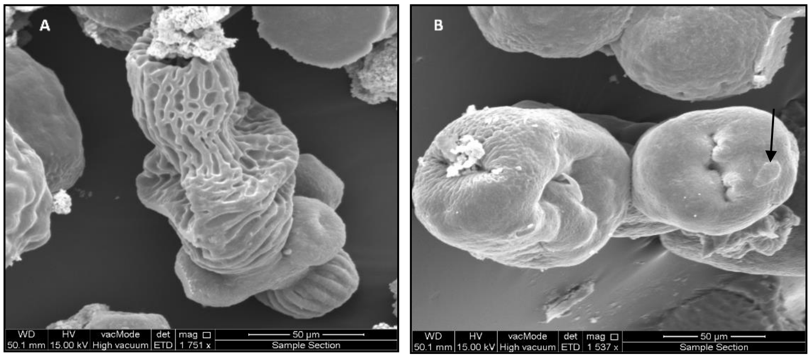
A
WD | HV | vacMode | det | mag
50.1 mm | 15.00 kV | High vacuum | ETD | 1 751 x
50 µm
Sample Section
B
WD | HV | vacMode | det | mag
50.1 mm | 15.00 kV | High vacuum | ETD | 1 537 x
50 µm
Sample Section

Fig. 3: A &B, protoscoleces incubated with Ag NPs, exhibit the existence of Ag NPs and the development of a bleb with morphological change. The Ag NPs accumulate like white constructions on the protoscoleces (Jalil et al. 2021).

microscopy examination, as illustrated in Fig. 3 (Jalil et al. 2021). Further, this study proposed the biocompatibility of Ag NPs as lysis of erythrocytes at various concentrations of Ag NPs was considerably ($P \leq 0.05$) lower than the value of the positive control.

In a recent paper, Hamad et al. (2022 a) performed the first in vivo evaluation of Ag NPs by *Z. spina-christi* leaves in BALB/c mice. In comparison to the untreated infected mice, the treated-infected mice displayed a variation in the liver hydatid cysts' appearance from hyaline to milky cloudy. This study also showed that Ag NPs administered orally to BALB/c mice (50, 100, 200, and 300 mg/kg) for acute short-term toxicity didn't cause any signs or adverse consequences, and no mortality. Moreover, the histopathological results in the intestine, kidneys and hepatic tissue of the animals treated with Ag NPs exhibited moderate histological impacts in contrast to the control ones, as illustrated in Fig. 4. The histological sections of the hydatid cyst in mice administered with 200 mg/kg showed rupture and destruction of the laminated and germinal layers. The findings also revealed that the cyst wall was destroyed, and vacuoles formed between the laminated and germinal layers.

Recent Studies of Green-based Synthesized Nickle Oxide Nanoparticle (NiO NPS)

In another work, *Z. spina-christi L.* leaf extract was used for synthesizing NiO-NPs. They pointed out that the green fabrication of NiO NPs possesses a powerful antioxidant

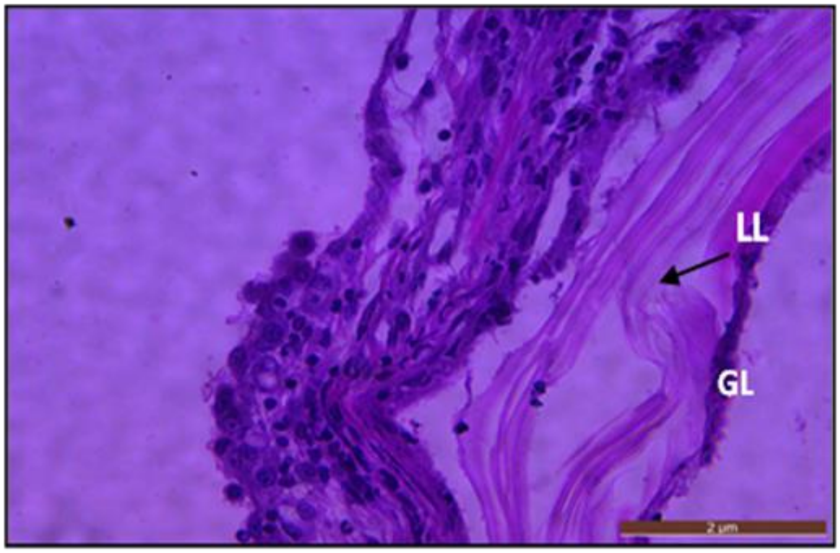

Fig. 4: Hydatid cysts from infected mice treated with AgNPs. The hydatid cysts layer is destroyed. The (LL) laminated layer converted to a loose layer with a vascular appearance. The LL seemed thin and separated from the (GL) germinal layer, stained with hematoxylin and eosin (Hamad et al. 2022 a).

action and minimal toxicity on the RBCs and seem hemocompatible. A high scolicidal action against *E. granulosus* with an efficient antibacterial action on *Escherichia coli* and *Staphylococcus aureus* was also reported (Shnawa et al. 2022b).

Recent Studies of Green-based Synthesized Copper Nanoparticles (Cu NPs)

In a study, the effects of copper nanoparticles (CuNPs) on hydatid cyst protoscoleces in vitro and ex vivo were investigated using *C. spinosa* extract and when mixed with albendazole (ALZ). Protoscoleces were exposed to different concentrations of CuNPs (250, 500, and 750 mg/mL) separately, as well in combination with Albendazole (200 mg/mL). At a concentration of 750 mg/mL in vitro, CuNPs displayed their maximum protoscolicidal activity, killing 73.3% of protoscoleces after 60 minutes of exposure. However, after being exposed for 10 min to 750 mg/mL of CuNPs together with 200 mg/mL of ALZ, protoscoleces died at a rate of 100% (Ezzatkhah et al. 2021). The results of this investigation demonstrated that CuNPs, particularly when combined with Albendazole, have strong protoscolicidal effects.

Furthermore, CuNPs at concentrations of 250, 500, and 750 mg/mL increased the activation of the caspase enzyme by 20, 32, and 36.1%, respectively, after 48 hours of treating protoscoleces. The outcomes also demonstrated that, even though the potential protoscolicidal mechanistic action of CuNPs is not fully clarified, one of the primary protoscolicidal mechanisms of CuNPs is inducing apoptosis through caspases (Ezzatkhah et al. 2021). Further, the mechanism of nanoparticles has been elucidated by previous studies as Mikami et al. (2013) explained the biological action of gold NPs by the creation of ROS, oxidative stress, DNA harm induction, and cell cycle effects. Moreover, this biological metal affects the tegument of the parasite and inhibits the synthesis of proteins (Kar et al. 2014). So the ultrastructural variations in the tegument are suggested to be a probable act of the NPs as an inhibitor of the synthesis of proteins (Napooni et al. 2019) Table 1 illustrated the updates on the effectiveness of biosynthesized nanoparticles against *E. granulosus* protoscoleces.

Proposed Mechanisms of Action

According to previous studies, the treated protoscoleces with plant material or bile acid-like chenodeoxycholic acid displayed decreased viability as well as morphological alterations, including the soma region contracting, bleb development, rostellar disorganization, loss of the parasite hooks, the obliteration of microtriches, and the formation of vesicles and lipid droplets (Shi et al. 2016; Shnawa et al. 2017). Additionally, since microtriches are directly linked to nutrition absorption, the structural changes of the tegument affect the microtriches, which may interfere with protoscoleces nutrition (Xing et al. 2016). The tegumental alteration, rostellar disorganization, loss of rostellum hooks, and the formation of numerous blebs on the tegument were among the changes in the protoscoleces. Previously, the same finding was seen in protoscoleces incubated in the existence of a variety of chemical substances, involving praziquantel and albendazole combined, flubendazole, thymol, and nitazoxanide. The nanoparticles exert their effects in various ways by disrupting DNA , preventing protein synthesis, and creating free radicals (Bajwa et al. 2022).

Table 1: Update on the effectiveness of biosynthesized nanoparticles against *E. granulosus* protoscoleces.

Nanoparticles	Plant	Concentrations	Model	Against	Activity	Reference
zinc oxide nanoparticles (ZnO NPs)	*Mentha longifolia* leaf extracts	100 ppm, 200 ppm & 400 ppm	*in vitro*	Protoscolices	The highest protoscolicidal effect was at 400 ppm., followed by 200, and 100 ppm. The scolicidal effect at 400 ppm after 150 min kills all the protoscolices.	(Shnawa et al. 2021)
zinc nanoparticles (ZnNPs)	*Lavandula angustifolia* extract	50, 100, and 200 µg/ml lonely and mixed with Albendazole (100 µg/ml)	*in vitro* and *ex vivo*	Hydatid cyst protoscolices	At 200 µg/ml, ZnNPs had the greatest scolicidal effect, killing 81.6% of protoscolices. After 10 minutes of mixing with ALZ at 200 g/ml, it damaged the protoscolices. At 200 µg/ml100% of protoscoleces were killed after 20 min in an *Ex vivo* trial. The dosage promoted the caspase-3 enzyme by 13.4%, 27.3%, and 34.8% at 50, 100, and 200 g/ml, respectively.	(Shakibaie et al. 2022)
zinc oxide nanoparticles (ZnO NPs)	*Ziziphus spina-christi* leaves	50,100, 200, 300, and 400 µg /ml	*in vitro*	protoscolices	At 400 µg / ml after 60 min of exposure,all protoscoleces were killed. Numerous morphological changes were detected in the protoscoleces. The lysis of erythrocytes at diverse concentrations of ZnO-NPs was significantly lower than the positive control.	(Shnawa et al. 2022 a)
Silver nanoparticles (Ag NPs)	*Ziziphus spina-christi* leaves	0.05, 0.1, 0.2, 0.3 & 0.4 mg/mL	*in vitro*	protoscolices	After 2 hours of treatment with Ag NPs , the entire killing of protoscoleces at 0.4 mg/ml was proved.	(Jalil et al. 2021)
Silver nanoparticles (Ag NPs)	*Ziziphus spina-christi* leaves	Mice were administered with 200 mg Ag NPs/kg. For toxicity experiments 50, 100, 200, and 300 mg/kg were tested.	*in vivo*	Hydatid cyst	The toxicity outcomes revealed that Ag NPs did not cause any adverse impacts or indications and no mortality in mice. The mice's liver, kidneys, and intestine revealed mild histological effects. The liver hydatid cysts in the treated-infected mice with Ag NPs changed from hyaline to milky cloudy in appearance.	(Hamad et al. 2022 a)
Silver nanoparticles (Ag NPs)	Leaves of *Ziziphus Spina-Christi* , *Piper nigrum*, and *Eucalyptus globulus*	0.025, 0.05, 0.1 and 0.15 mg/mL	*in vitro*	protoscolices	Scolicidal action against *E. granulosus* was detected at 47.8 % after 45 min.	(Salih et al. 2020)
Nickle oxide nanoparticles (NiO NPS)	*Ziziphus spina-christi* leaves	0.1, 0.2, 0.3, and 0.4 mg/mL	*in vitro*	protoscolices	Levels of 400, 300, and 200 µg/mL of NiO NPs presented more quick results with non-significant differences, killing almost all protoscolices after one hour.	(Shnawa et al. 2022 b)
Copper nanoparticles (CuNPs) only and mixed with Albendazole	*Capparis spinosa* Fruits	250, 500, and 750 mg/mL separately and mixed with Albendazole of (200 mg/mL)	*in vitro* and *ex vivo*	protoscolices	At 750 mg/mL in vitro, CuNPs had the highest protoscolicidal action and caused 73.3% mortality after 60 minutes of exposure. After 10 minutes, CuNPs mixed with 200 mg/mL of ALZ have 100% mortality. Caspase activity was also induced by 250, 500, and 750 mg/mL of CuNPs.	(Ezzatkhah et al. 2021)

The ability of hydatid fluid antigens to cause apoptosis in surrounding tissue was demonstrated by a recent study that revealed a high percentage of caspase-3 in mouse liver samples (Hamad et al. 2022b). It is a parasite strategy for surviving and managing the immune response, according to Hussein et al. (2020). and found that the parasite could stimulate apoptosis in hepatic and spleen tissues after the induction of a cystic echinococcosis mouse model. Immunohistochemistry analysis also demonstrated that cytokeratin and caspase3 levels were higher in echinococcus tissues than in healthy tissues. According to Yang et al. (2022), Hepatic echinococcosis causes the liver to express high levels of cytokeratin and other molecules related to apoptosis, which indicate liver damage, as shown in Fig 5.

Conclusion

According to previous research, the use of nanoparticles along with albendazole eradicated the parasite within in vitro, ex vivo, and in vivo; nevertheless, additional research is needed to evaluate the efficacy and safety of these NPs as a promising scolicidal agent in the preclinical setting (in animals) and clinical setting, not only when administered intraperitoneally but also via other routes like oral administration. The induction of apoptosis through caspases is one of the main mechanisms, even though the potential protoscolicidal mechanisms of NPs are not fully understood. But it's important to understand how these biogenic NPs work against CE at the cellular and molecular levels.

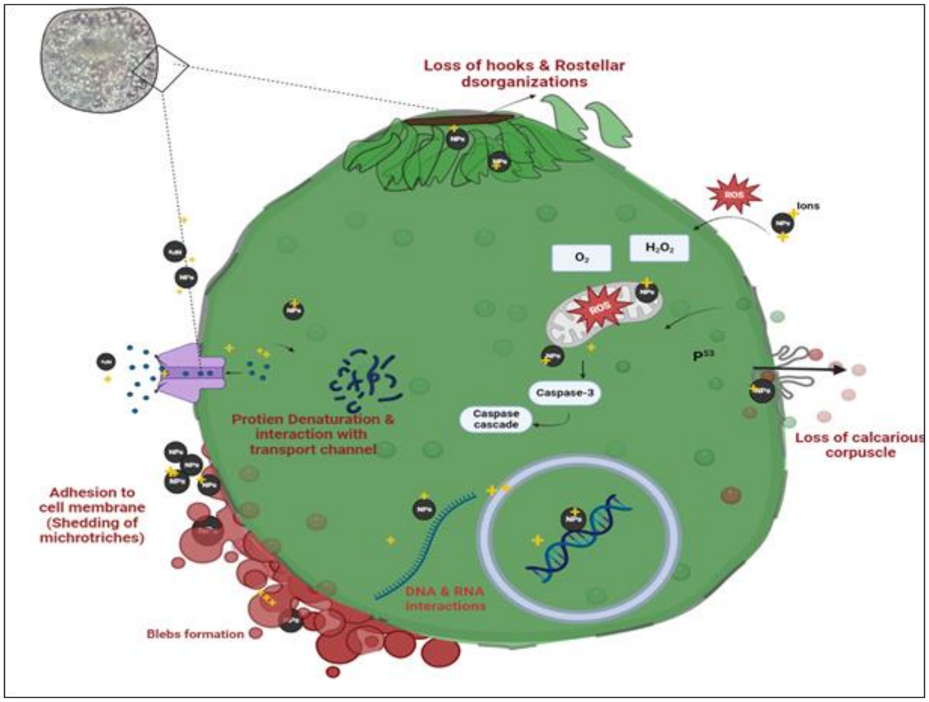
Loss of hooks & Rostellar dsorganizations
Ions
ROS
O_2
H_2O_2
ROS
P^{53}
Caspase-3
Caspase cascade
Loss of calcarious corpuscle
Protien Denaturation & interaction with transport channel
Adhesion to cell membrane (Shedding of michrotriches)
DNA & RNA interactions
Blebs formation

Fig. 5: The potential mechanism of green synthesized nanoparticles against hydatid cyst protoscolices.

These may offer a fresh perspective on NP targets and possibly the opportunity to develop a new, more potent drug for human CE on an immediate basis.

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10

Blunt Antibiotic Weapons Against Mastitis

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INTRODUCTION

Mastitis or simply put as the inflammation of mammary glands, is a global challenge faced by dairy farmers that results in major economic losses for the dairy industry (Hogveen et al. 2011; Gomes and Henriques 2016; Jamali et al. 2018). Mainly, these losses are attributed to reduced milk quality, lower milk yield, and increased use of drugs, particularly antibiotics that is further linked to some major problems like the antibiotic resistance phenomenon (Miller et al. 1993; Azooz et al. 2020). Some other economic losses as a consequence of mastitis include the increased expense of veterinary services, increased culling rate, and sometimes even death of the animals (Kumar et al. 2010). On an average, a farmer loses around $147 per cow per year due to mastitis (Hogeveen et al. 2019). Mastitis is a term used to indicate inflammation of the udder in mammals. More accurately the inflammation of udder parenchyma is referred as mastitis. It is characterized by usual inflammatory signs seen on the udder. These signs can include edema, swelling, increased temperature of the udder and abnormal physical and chemical characteristics of milk being produced by the affected udder (Radostitis et al. 2006; Paramasivam et al. 2023). Mastitis is classified according to the appearance of clinical signs as clinical or subclinical (Antanaitis et al. 2022). Furthermore, clinical mastitis can be acute, sub-acute, or per-acute depending on the severity of clinical signs (Kibebew 2017). The subclinical status of mastitis is the main hurdle in its control as it shows no visible signs (Abebe et al. 2016). Although, several efforts like the "Five-point-plan" and antibiotic therapy have been put into practice to control mastitis (Hossain et al. 2017; Breen 2019). However, recently the misuse of antibiotics in animals has led to the development of antibiotic resistance in pathogens of mastitis making it even more difficult to cure (Gomes and Henriques 2016). The rise of antibiotic resistance hence needed a remedy in the form of some other alternatives besides conventional antibiotics and the best of these turned out to be plant extracts (Zaatout 2022). An important reason for using plant extracts was their easy availability and affordability (Rakholiya et al. 2013; Yusuf et al. 2022). This chapter aims to explain antibiotic resistance in some important pathogens of mastitis along with its causes, effects, and treatment.

Antibiotic Resistance

Antibiotic resistance is a hot topic of several health security debates among concerned authorities of various countries. Antibiotics are our only chance against pathogenic bacteria and in recent years, widespread misuse of these drugs has also played a role in making our only weapon of defense, blunt against enemies (Oliver and Murinda 2012). The only way to undo our outrageous mistake is to closely monitor the surveillance of healthcare policies and practices by policymakers, researchers, and prescribers to mitigate the complex task of antibiotic resistance in the new era of science (Velez and Sloand 2016). Antibiotic resistance in microbial germs is a complex provocation worldwide. This villain isn't just another healthcare challenge of science but a baddie that comes with scoundrels that go by names of "high morbidity" and "high mortality" (Akova 2016). Antimicrobial resistance is also a matter of grave concern for health professionals and veterinarians alike because it is suspected to be transferable from animals to humans through the food chain. This is the focus of modern research related to antimicrobial resistance (Piddock et al. 1996).

Antibiotic Resistance against Mastitis

Beta-lactams have been used for a long time for the treatment of mastitis worldwide. Recently, there has been a sharp decline in the efficiency of beta-lactams due to the synthesis of beta-lactamase enzymes by bacteria to resist the effect of these drugs. Beta-lactamase is encoded in the bacterial genome by blaZ (Olsen et al. 2006). Some bacteria also have

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a mecA genome that codes for low-affinity penicillin-binding proteins (PBP2a). These proteins mediate methicillin or oxacillin resistance mechanisms to nullify the effectiveness of beta-lactams (Sawant et al. 2009). Bacterial pathogens also have natural defense systems against antimicrobial drugs. An example is the leading causative agent of mastitis is *Staphylococcus (S.) aureus*. It has a lower cure rate as compared to other mastitis agents. This lower cure rate is supposed to be because of the biofilm-forming abilities of this bacteria (Taponen and Pyörälä 2009). Biofilm-forming ability reduces the susceptibility of *S. aureus* against various antimicrobial drugs by reducing the diffusing efficiency of these drugs inside the biofilm matrix. Besides, providing *S. aureus* with antimicrobial resistance this biofilm also grants it enhanced bacterial adhesion ability to colonize mammary cells more easily and helps it evade harsh conditions and attempts of phagocytosis by the host's immune cells. Biofilm also increases the persistence of infections caused by *S. aureus*. There is an ica locus present in the genomes of *S. aureus* and *S. epidermidis*. This locus encodes icaA and icaD genes that are majorly responsible for biofilm formation (Arciola et al. 2001). Antimicrobial resistance is also a public health hazard for humans along with animals. It is responsible for low cure rates against bacterial diseases despite the use of antimicrobial drugs. Several strains of *S. aureus* recently collected from mastitis-affected udder milk samples are found to be resistant to various antimicrobial drugs (Table 1). These drugs include gentamicin, penicillin-G, streptomycin, ampicillin, oxytetracycline, and ciprofloxacin (Kumar et al. 2011; Singh et al. 2018).

Methicillin-Resistant *Staphylococcus aureus* (MRSA)

S. aureus is a coccoid bacterium of Gram-positive nature. Being a coccoid organism means its cells are arranged in a grape-like irregular pattern. Usually, they reside in humans and animals as normal flora. It is found widely among humans with 30–40% of adults being asymptomatic carriers. It is considered a major culprit for infections in humans. It can produce a wide variety of infections in humans ranging from skin infections of and food poisoning of mild nature up to severe infections that can be fatal in the end. The discovery of methicillin-resistant *S. aureus* was primarily reported in 1961 shortly after the launching of antibacterial agents or antibiotics for clinical use (Conly and Johnston 2002; Taiwo 2011; Onemu and Ophori 2013; Kobayashi et al. 2015; Kong et al. 2016).

After that these pathogens began to multiply rapidly starting a global epidemic of Methicillin-resistant *S. aureus* (MRSA). This wave was felt in both community centers and healthcare systems (Weinstein and Fridkin 2001; Appelbaum 2006; Loomba et al. 2010). Isolates of MRSA from Denmark and the UK during the early phase of the 1960s were the very first indicators of an upcoming epidemic. The methicillin-resistant clone of *S. aureus* was introduced at that and since then it has emerged as a pathogen of utmost importance in human medicine that gives the premonition the era of antimicrobial resistance (Pinho et al. 2001; Lee 2003; Harkins et al. 2017). Despite methicillin being out of practice, and being replaced by isoxazolyl penicillins, particularly flucloxacillin in the UK, it is only out of the prescriptions for patients. Still, it has been a nightmare for the researchers. The acronym MRSA has still stayed as a nightmare reminding people of the upcoming dark times (Johnson 2011).

MRSA terminology is being used to characterize antibiotic resistance of bacteria to penicillins, cephalosporins, and carbapenems. Later, these bacteria have the tendency to develop resistance to quinolones, aminoglycosides, and macrolides (Okwu et al. 2012; Arunkumar et al. 2017; Adhikari et al. 2017). The origin of MRSA was a consequence of Staphylococcal Cassette Chromosome mec (SCCmec) genes being acquired by methicillin-susceptible *S. aureus* (MSSA). These genes, SCCmec, harbored the mecA gene. It was the gene that the penicillin-binding protein (PBP2a) encoded in itself. It is the main culprit that conferred resistance to all bacteria against β-lactam antibiotics (Rodríguez-Noriega et al. 2010; Arunkumar et al. 2017; Otto 2017; Sit et al. 2017).

SCCmec genes also contain the gene complex of cassette chromosome recombinases (ccr). The ccr genes mediate the integration and excision of SCCmec from the methicillin-resistant bacterium's chromosomes and into the chromosomes of susceptible bacteria, as it is composed of ccrC or a pair of ccrA and ccrB, and encode recombinases. The genes of ccr and surrounding genes make the complex of ccr genes. SCCmec contains a few genes and various other mobile genetic elements. These elements include insertion sequences, transposons, and plasmids along with ccr and mec gene complexes (Milheiriço et al. 2007; Okwu et al. 2016). Several different types of SCCmec numbered around eleven (I-XI) and some allotypes of the ccr gene complexes that are up to five (ccrAB1, ccrAB2, ccrAB3, ccrAB4, and ccrC) have been reported. Usually, hospital-acquired MRSA (HA-MRSA) is caused by SCCmec types I, II, III, VI, and VIII, while community-acquired MRSA (CA-MRSA) includes types IV, V, and VII while livestock-associated MRSA (LA-MRSA) includes types IX, X, and XI (Amirkhiz et al. 2015; Okwu et al. 2016).

Methicillin-resistance gene expression in *S. aureus* is commonly controlled and regulated by the mecI or blaI gene. These mecI and blaI gene repressors are under the control of the mecRI and blaRI transducers (Loomba et al. 2010). MRSA remains one of the major public health issues worldwide. It is also a therapeutic challenge as the treatment is costly due to the scarce availability of effective antibiotic drugs and their ever-increasing prices. The ever-varying epidemiological patterns of MRSA infections, changing resistance to antibiotics of common use, and involvement of community centers and hospitals in spreading infections have influenced the utilization and clinical results of presently available antibiotic drugs (Rodvold and McConeghy 2014).

Table 1: Antibiotic resistance (%) of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Streptococcus agalactiae* (Singh et al. 2018).

No.	Antibiotics	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Streptococcus agalactiae*
1.	Chloramphenicol	31.03%	0%	0%	28%
2.	Co-trimoxazole	13.79%	60%	28%	13.9%
3.	Tetracycline	34.48%	37.03%	36%	67.44%
4.	Gentamicin	41.37%	0%	0%	4.6%
5.	Ofloxacin	0%	63%	40%	0%
6.	Erythromycin	31.03%	7.5%	76%	41.86%
7.	Amoxicillin	93.10%	100%	100%	93.03%
8.	Cephalexin	20.68%	7.5%	4%	23.25%
9.	Sparfloxacin	0%	33.33%	60%	9.3%
10.	Ciprofloxacin	0%	29.62%	24%	4.6%
11.	Vancomycin	31.03%	66%	36%	34.88%
12.	Teicoplanin	0%	66%	100%	30%
13.	Doxycycline	0%	26%	36%	90.69%
14.	Azithromycin	0%	0%	0%	0%
15.	Gatifloxacin	31.03%	29.62%	56%	29.03%

Vancomycin-Resistant *Staphylococcus aureus* (VRSA)

Vancomycin is an antibacterial agent and it works by inhibiting the production of bacterial cell walls. As a cell wall inhibitor, vancomycin performed its function by binding with precursors of a bacterial cell wall named Dalanyl-D-alanine C terminus. As a result of this binding, vancomycin stops the crosslinking through a process of transpeptidation. In more detail, in the process of inhibition of the production of the bacterial cell wall, vancomycin performs its function extracellularly and stops the biosynthesis of late-stage peptidoglycan. This extracellular activity of vancomycin results in the accumulation of precursors named UDP-linked MurNAc pentapeptide. The structure of the vancomycin complex basically consists of a number of hydrogen bonds present between the vancomycin peptide component and the bacterial cell wall residue D-Ala-D-Ala. So, if there is any condition of any other process which will disturb the binding of vancomycin drug with the D-Ala-D-Ala residue, it will affect the end results, for instance, the potency of the drug will decrease (Conly and Johnston 2002; Howden et al. 2010). The use of vancomycin against MRSA is increasing day by day and it is used in a huge amount for the treatment of MRSA, the outcomes of this are the emergence of vancomycin-intermediate and vancomycin-resistance *S. aureus* (VISA and VRSA) (Dhanalashmi et al. 2010). This situation is very alarming as *S. aureus* is a potential cause of infections in both hospitalized and non-hospitalized patients of the medical community (Weinstein and Fridkin 2001).

Many classes of *S. aureus* have developed resistance against vancomycin in different parts of the world. The main three classes which developed resistance include vancomycin-intermediate *S. aureus* (VISA), heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and the last one is vancomycin-resistant *S. aureus* (VRSA) (Appelbaum 2007). The first hospital stain of vancomycin-resistant *S. aureus* (VRSA) was reported in 2002 in a hospital in the United States (CDC 2002). When the trials are started to collect the vanA gene from vancomycin-resistant enterococci, the outcomes are in the form of the bursting of the vancomycin-resistant strain of *S. aureus* (VRSA) and the MIC value of vancomycin with this was higher than 16ug/mg (Chang et al. 2003; Howden et al. 2010; Gardete and Tomasz 2014).

Novel Strategies to Mitigate Resistant *S. aureus*

Different strategies can be wielded as a weapon to strike off the hydra of antibiotic resistance in the causative microbes of mastitis which include novel antibiotic development, new drug development, nanoparticles, and some herbal extracts too. In the past two decades, many research studies have been done on the herbal way to tackle methicillin-resistant *S. aureus* (MRSA). These herbal products are derived from various parts i.e., wood, leaves, stems, roots, etc, of specific plants with the help of specific extraction agents like ethanol or methanol. These products have shown specific antimicrobial properties which enable them to tackle Methicillin-resistant *S. aureus* (Table 2) (Shopsin et al. 1999; Yang-Hwei et al. 2008; Hamal et al. 2009; He et al. 2014; Jesline et al. 2015; Natarajan et al. 2015; Zaatout 2022).

Different nanoparticles withhold antimicrobial capacities of various intensities depending upon the rival microbe, population of the microbes, resistance properties of the microbe and the environment of the microbial population where the interaction occurs between the two (Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013; Hozyen et al. 2019). These nanoparticles can be used against various resistant causative microorganisms of both clinical and subclinical mastitis (Table 3).

Conclusion

Antimicrobial resistance is a burning issue around the globe these days. As there is a lack of novel drugs and the development of new antibiotics isn't happening that often, antimicrobial resistance knocks down the only shield held

Table 2: Botanical products that have antimicrobial properties to mitigate MRSA.

No.	Plant name	Family	Part of the plant used	References
1.	*Acacia catechu* (L.f.) Willd	Fabaceae	Wood	(Voravuthikunchai and Kitpipit 2005; CABI 2018a)
2.	*Impatiens balsamina*	Balsaminaceae	Leaves	(Voravuthikunchai and Kitpipit 2005; CABI 2018b)
3.	*Walsura robusta*	Meliaceae	Wood	(Voravuthikunchai and Kitpipit 2005; Arefin et al. 2011)
4.	*Butea monosperma Lam.*	Fabaceae	Leaves	(Sahu and Padhy 2013)
5.	*Acacia albida* Del.	Fabaceae	Stem Bark	(Aliyu et al. 2008)
6.	*Anchomanes difformis* Engl.	Araceae	Roots	(Aliyu et al. 2008)
7.	*Boscia senegalensis* Del.	Capparidaceae	Roots	(Aliyu et al. 2008)
8.	*Moringa oleifera* Lam.	Moringacceae	Leaves	(Aliyu et al. 2008)
9.	*Mormodica basalmina* Linn.	Cucurbitaceae	Whole Plant	(Aliyu et al. 2008)
10.	*Nymphaea lotus* Linn.	Nymphaeaceae	Leaf	(Akinjogunla et al. 2010)
11.	*Pavetta crassipes* K. Schum.	Rubiaceae	Roots	(Aliyu et al. 2008)
12.	*Phyllanthus amarus* Schum. Thonn.	Euphorbiaceae	Roots	(Aliyu et al. 2008)
13.	*Vernonia blumeoides* Hook. f.	Asteraceae	Aerial part	(Aliyu et al. 2008)
14.	*Machilus salicina* Hance.	Lauraceae	TBL	(Zuo et al. 2012)
15.	*Selaginella tamariscina* (Seauv.) Spring.	Selaginellaceae	Whole plant	(Zuo et al. 2012)
16.	*Celastrus orbiculatus* Thunb.	Celastraceae	Vane	(Zuo et al. 2012)
17.	*Carex prainii* C.B. Clarke	Cyperaceae	Whole plant	(Zuo et al. 2012)
18.	*Embelia burmf.*	Myrsinaceae	Leaves	(Zuo et al. 2012)
19.	*Withania somnifera L.*	Solanaceae	Roots & Leaves	(Heyman et al. 2009; Uddin et al. 2012; Nefzi et al. 2016)

Table 3: Nanoparticles that have antimicrobial properties to mitigate the causative & resistant bacteria of subclinical and clinical mastitis.

No.	Microbe	Nanoparticle	Reference
1.	*S. aureus*	Zinc Nanoparticles	(Namasivayam et al. 2015)
		Titanium Nanoparticles	(Shopsin et al. 1999; Yang-Hwei et al. 2008; Hamal et al. 2009)
		Iron Oxide	(Behera et al. 2012; Ismail et al. 2015; Masadeh et al. 2015)
		Cobalt Iron Oxide	(Kooti et al. 2015)
		Copper Nanoparticles	(Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013)
		Magnesium Oxide	(Jeong et al. 2007)
		Calcium Oxide	(Jeong et al. 2007)
2.	*E. coli*	Zinc Nanoparticles	(Namasivayam et al. 2015)
		Titanium Nanoparticles	(He et al. 2014; Jesline et al. 2015; Natarajan et al. 2015)
		Iron Oxide	(Behera et al. 2012; Ismail et al. 2015; Masadeh et al. 2015)
		Silicon & Silver Oxide	(Kooti et al. 2015)
		Copper Nanoparticles	(Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013)
		Magnesium Oxide	(Jeong et al. 2007)
		Calcium Oxide	(Jeong et al. 2007)
3.	*Klebsiella pneumoniae*	Copper Nanoparticles	(Esteban-Cubillo et al. 2006; Theivasanthi and Alagar 2011; Ramyadevi et al. 2012; Subhankari and Nayak 2013)
		Iron Oxide	(Behera et al. 2012; Ismail et al. 2015; Masadeh et al. 2015)

against any upcoming disease outbreak or pandemic. There has been a massive rise in the antimicrobial resistance in the causative agents of subclinical and clinical mastitis, among which Methicillin-resistant *S. aureus* (MRSA) and Vancomycin-resistant *S. aureus* (VRSA) are the most common or prevalent ones. Different strategies can be wielded as a weapon to strike off the hydra of antibiotic resistance in the causative microbes of mastitis which include novel antibiotic development, new drug development, nanoparticles, and some herbal extracts too. In the past two decades, many research studies have been done on the herbal way to tackle methicillin-resistant *S. aureus* (MRSA). These herbal products are derived from various parts i.e., wood, leaves, stems, roots, etc., of specific plants with the help of specific extraction agents like ethanol or methanol. These products have shown specific antimicrobial properties which enable them to tackle Methicillin-resistant *S. aureus*. Different nanoparticles withhold antimicrobial capacities of various intensities depending upon the rival microbe, the population of the microbes, the resistance properties of the microbe, and the environment of the microbial population where the interaction occurs between the two. These nanoparticles can be used against various resistant causative microorganisms of both clinical and subclinical mastitis.

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11

Chlamydiosis in Human

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INTRODUCTION

Chlamydiae species are the causative agent for the disease chlamydiosis. A according to their antigenic content, intracellular inclusions, sulfadiazine susceptibility, plus the ability to cause sickness, three forms of chlamydiae that infect humans are *C. trachomatis*, *C. pneumoniae* and *C. psittaci* belonging to the genus *Chlamydia* (Carroll et al. 2016). Therefore, different chlamydiae may infect animals, but seldom or never humans. All chlamydiae share a group antigen, have a similar morphology, and reproduce inside the cytoplasm of their host cells through a certain growing cycle (Al-Barzanji 2020). Chlamydiae are the gram-negative bacteria that lack systems for generating metabolic energy and are unable to manufacture adenosine triphosphate (ATP). Chlamydiae are strictly intracellular pathogens due to the restriction inside the cells. Thus, the host cell provides intermediates that are rich in energy (Tille 2017). Cervix inflammation, pelvic inflammatory disease (PID), urethral inflammation, inflammation of the epididymis, LGV and inflammation of the rectum are the sexually transmitted diseases caused by *C. trachomatis*. When it infects pregnant women, it can also lead to fetal conjunctivitis and eosinophilia pneumonia (Workowski et al. 2021). Numerous upper and lower respiratory tract infections are produced by *C. pneumoniae*. About 5% of acquired pneumonia is caused by common and atypical pneumonia pharyngitis, which resembles *Mycoplasma pneumoniae* (Hammerschlag et al. 2015). Dealing with birds such as parrots, pigeons, and different household avian species can lead to an acquisition of *C. psittaci*. Psittacosis may be unnoticed or moderate, but it has also been reported to cause severe pneumonia and sepsis, both of which have a higher fatality rate (Zhang et al. 2022).

History of *Chlamydia*

Conjunctival scraping samples from trachoma patients were found to include intracytoplasmic inclusions in 1907 by Halberstaedter and von Prowazek, two of Albert Neisser's assistants leading to the discovery of the infectious agent. These inclusions suggested the term "Chlamydozoa" since they were draped around the nucleus (Greek chlamys meaning a cloak). This supposed organism believed for protozoa, then a virus, but it was actually a bacterium adapted to live within cells. (Haider 2009; Taylor-Robinson 2017).

During a human psittacosis outbreak in 1930, "Bedson and Western" identified a minute basophilic particle from the blood and tissues of affected birds and humans; than in the same year, the Lymphogranuloma Venereum (LGV) causative agent was isolated from human tissue. These particles were categorized as viruses in the psittacosis-LGV group because they were not inhibited by bacterial filters and could not be cultivated on artificial media (Al-Barzanji 2020). Over the course of the next 50 years, numerous attempts to cultivate these uncommon organisms were made, but it wasn't until 1957 that the trachoma factor was isolated from chicken-embryo yolk sacs after inoculating them with material from affected human eyes (Jones et al. 1964; Taylor-Robinson 2017).

For almost ten years following their initial isolation, chlamydiae were initially believed to be large viruses due to their obligate intracellular existence. It wasn't until chlamydiae were finally discovered to be a closely related group of gram-negative intracellular bacteria that they had been given their own order, Chlamydiales, one family, *Chlamydiaceae*, and a single genus, *Chlamydia* (Moulder et.al. 1984; Satterwhite and Douglas 2013). The infection was not recognized as a sexually transmitted disease until 1976 because chlamydial disease was first identified in the eye and has a wide variety of symptoms or lack of symptoms that are identical to other diseases or syndromes (Schachter et al. 1976; Balogh 2014).

Etiology

Members of the Chlamydiales order and *Chlamydiaceae* family are the *Chlamydia* spp. *Chlamydiaceae* members had been first grouped by Everett et al. (1999). Based on variations in phenotypic, 16S ribosomal ribonucleic acid (rRNA), and 23S rRNA, *Chlamydia* was split into two genera, *Chlamydia* and *Chlamydophila*. Recent taxonomy of all presently identified *Chlamydiaceae* species into a single genus, the genus *Chlamydia*, has proposed by Sachse et al.

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(2015). Because of their tiny size that may pass through 0.45µm filters, the *Chlamydiaceae* were previously misidentified for viruses. The Chlamydiae are obligate intracellular parasites that depend on their eukaryotic hosts' metabolic resources to give energy for their own metabolism for development and copying by giving it high-energy substances like adenosine triphosphate (ATP) (Balogh 2014; Arif et al. 2020).

The outer cell wall of chlamydiae is similar to that of gram-negative bacteria. This bacterium's center dense core is encircled by a cytoplasmic membrane and a double-layer outer membrane, despite the absence of the stiff peptidoglycan layer seen in the majority of other bacteria. All members of the family share a lipopolysaccharide (LPS) in their cell walls. There is very little endotoxin action in LPS. The major outer membrane protein (MOMP), which is specific to each species, is an essential structural element of the outer membrane (Arif et al. 2022).

There are 18 serological variations (also known as serovars) in *C. trachomatis* due to variable sections in the gene encoding this protein (Witkin et al. 2017). The *C. pneumoniae* MOMP is homogenous, and only one serovar has been described, in contrast to the *C. psittaci* MOMP, which has similar variable areas. All members of the *Chlamydiaceae* family possess OMP 2, a second highly conserved outer membrane protein. This cysteine-rich protein is in charge of creating the many disulfide cross-links that give elementary bodies their stability (Schlossberg 2015).

Morphology and Developmental Cycle

Chlamydiaceae, unlike other bacteria, have a distinct developmental cycle that results in the formation of metabolically active, non-infected shapes termed reticulate bodies (RBs) and metabolically inactive, infectious forms called elementary bodies (EBs). "EB" is a tiny, 0.3 µm diameter cell that resembles spores in size and has an electron-dense nucleoid. The EBs can connect to receptors on host cells and induce absorption by the infected cell despite the fact that they cannot reproduce and are resistant to many harsh environmental stressors (Haider 2009; Arif et al. 2022). In this intracellular site, EBs changes into RBs, a replicating, metabolically active form without an electron-dense nucleoid that is roughly 0.5–1 µm in size. This type is osmotically weak since the vast cross-linked proteins are not present in RBs, but they are safeguarded by their intracellular location (Schlossberg 2015).

There are five main stages in *Chlamydia* developmental cycle. Following the EB's attachment and uptake, the metabolically inactive EB is then taken up by metabolically active RB. Then the RBs begin to multiply and proliferate, maturing from noninfectious RB into infectious EB. Finally, this host cell releases new EBs (Whittum and Hudson 2005; Al-Barzanji 2020).

An acute infection occurs with the EB's attachment to the surface of the eukaryotic cell. Surface microvilli are the site where binding occurs most frequently. The attachment to these sites may facilitate a quick and effective entry since the base of the microvilli serves as active transport sites for extracellular components into the host cells (Escalante et al. 1998).

Upon binding, the EBs is internalized in small, endocytic vesicles known as inclusions, however the method by which the EBs adhere to and enter cells is yet unknown. Since then, a number of unique mechanisms have been suggested (Wyrick et al. 1989). These mechanisms include microfilament-dependent phagocytosis, pinocytosis in non-clathrin-coated pits, and receptor-mediated endocytosis in clathrin-coated pits (Prain and Pearce 1989).

Two hours after infection, the new kinds of inclusions successfully avoid fusion with cellular lysosomes, and EBs begins to develop into RBs. Eight hours after infection, these RBs travel to the edge of the inclusion and begin replicating. The inclusion quickly fills in and grows in size as the RBs grow. The RB offspring re-condense back into infectious EBs that is metabolically dormant after a period of 24-48 hours. When the host cell ruptures or exocytosis occurs 48 to 72 hours after infection, the EB are kept in the inclusion lumen until they are freed and can then invade other cells. The range of the developmental cycle diverges depending on the species (Moulder 1991).

Classification

Human-infecting Chlamydiae are classified to 3 species, *C. trachomatis*, *C. pneumoniae,* and *C. psittaci*, based on their antigenic content, susceptibility to sulfadiazine, intracellular inclusions and ability to cause disease (Carroll et al. 2016). The 3 *Chlamydia* species infecting human and their characteristic features have been enlisted in Table 1.

Chlamydia trachomatis

General Characteristics

Humans are virtually entirely infected with *C. trachomatis*, which causes a number of clinical symptoms. 18 distinct serovars of *C. trachomatis* have been identified, each of which is linked with a different primary clinical symptom based on MOMP antigenic differences. The main way that *C. trachomatis* infections spread from person to person is through dealings with infected secretions. Some diseases, including neonatal pneumonia or inclusion conjunctivitis, are passed from mother to the child after birth (Batteiger and Tan 2015; Witkin et al. 2017).

Pathogenesis

Humans are the primary host for *C. trachomatis'*. Whereas the processes causing the tissue damage and inflammation produced on by *C. trachomatis* are not well known. The chlamydial species may infect a widespread domain of cells,

Table 1: *Chlamydia* with various features that cause human disease.

Property	*Chlamydia trachomatis*	*Chlamydia pneumoniae*	*Chlamydia psittaci*
Inclusion morphology	Circular and vacuolar	Circular and dense	Large, erratic in shape and dense
Glycogen in inclusions	Yes	No	No
Elementary body morphology	Circular	Circular and pears-shaped	Circular
Susceptible to sulfonamide	Yes	No	No
Plasmid	Yes	No	Yes
Serovars	15	1	≥4
Natural host	Humans	Humans and animals	Birds
Mode of transmission	Mother to child, person to person	Airborne, person to person	Airborne, bird excreta to humans
Major diseases	Trachoma, LGV ,STDs, infant and pneumonia	Pneumonia, pharyngitis, bronchitis and sinusitis	Psittacosis, pneumonia

including smooth muscle cells, monocytes and epithelial cells of the mucosa and blood vessels. The chlamydial EB enters the host cell by phagocytosis and is stored in a vacuole that does not combine with a lysosome, allowing the organism to survive inside the cell and avoid the host immune response (Darville and Hiltke 2010).

Apoptosis (organized cell death pathways) can be activated or deactivated by chlamydiae in infected host cells. In the acute infection process, the organism negatively regulates inflammation by causing surrounding host damaged cells, whereas in the event of chronic infection, the organism maintains the host cell's existence by blocking apoptosis, enabling it to persist (Wyrick 2010).

The Aspects of Disease

A- Trachoma

The Ebers Papyrus, which was written in Egypt 3800 years ago, provides a detailed description of the ancient eye disease "trachoma". The chronic keratoconjunctivitis causes blindness and scarring after starting as a severe inflammatory alteration in the conjunctiva and cornea (Tille 2017). The infection spread through the contact with infectious fluids on hands, towels, or flies. Clinical trachoma is related to the *C. trachomatis* serovars A, B, Ba, and C (Batteiger and Tan 2015).

Clinical Findings

Chlamydial conjunctival incubation lasts for 3 to 10 days. The contact of organisms with contaminated secretions, which may be found on clothing, fingers, or those carried by flies resulting in the infection (Satterwhite and Douglas 2013).

Lacrimation, mucopurulent discharge, conjunctival hyperemia, and follicular enlargement are the early signs of trachoma. Epithelial keratitis, sub epithelial infiltrates, and the expansion of limbal capillaries into the cornea are all visible when the cornea is examined under a microscope (pannus). As the pannus extends downward across the cornea, there are scarring of the conjunctiva, eyelid deformities (entropion, trichiasis) and an added insult caused by eyelashes sweeping across the cornea (trichiasis). With secondary bacterial infection, loss of vision progresses over a period of years. There are, however, no systemic symptoms or signs of infection (Carroll et al. 2016).

B- Lymphogranuloma Venereum

A sexually transmitted infection known as lymphogranuloma venereum (LGV), which is uncommon in North America but it's quite common in Africa, Asia, and South America. In Europe, the infection is resurfacing primarily among male homosexuals. Unlike *C. trachomatis* serovars A through K, which allow the mucosa to extend to the localized lymph nodes, *C. trachomatis* serovars L1, L2, L2b and L3 are invasive and produce LGV (DeVries et al. 2010; Lefebvre 2014).

Clinical Findings

Through few days to several weeks a small evanescent papule or vesicle may appear anywhere on the external genital organs, rectum and anus (Witkin et al. 2017). A primary vaginal lesion that manifests itself briefly at the location of the original infection identifies the disease. Particularly in individuals who are female, this lesion is usually tiny and impossible to see. The inguinal lymph nodes are commonly detected by the second stage of acute lymphadenitis, which causes them to swell and mat together, creating a significant region of groin swelling referred as the bubo (Dewart et al. 2018). During this stage, an infection may spread locally and result in granulomatous proctitis or it may spread systemically and produce fever. A small percentage of patients (more women than men) develop a chronic third stage of the disease, which results in the production of vaginal hyperplasia, rectal fistulas, rectal strictures, draining sinuses, and other symptoms (Chan et al. 2016).

C- Oculogenital Infections

Particularly in industrial countries, *C. trachomatis* serovars D-K cause a sexually transmitted disease and may also lead to an eye infection (inclusion conjunctivitis). Both adults and infants could get acute inclusion conjunctivitis from *C.*

trachomatis. Infection is transmitted, when infected vaginal discharges are touched into the eyes, or when a newborn is delivered through the birth canal (Workowski et al. 2021).

Auto-infection may happen but infrequent cases have been reported. Additionally, the organism can be obtained through hot tubs that lack proper chlorination, swimming pools, or by exchanging eye makeup. The symptoms of inclusion conjunctivitis include dilated eyes and a purulent secretion. It does not cause adult blindness, unlike trachoma (or newborns) (Carroll et al. 2016).

C. trachomatis can occasionally lead in epididymitis and nongonococcal urethritis in high sexually energized males. *C. trachomatis* also causes urethral inflammation, cervicitis, and pelvic inflammation in females, which can induce infertility and raise the probability of an ectopic pregnancy (Falasinnu et al. 2016).

Asymptomatic carriage in both sexes may remain, frequently for months. Many genital chlamydial infections in both sexes were silent or difficult to identify by clinical standards. Up to 50% of men and 70% to 80% of women with chlamydial genital tract infections are observed to be asymptomatic (Tille 2017).

D- Perinatal Infections

Inclusion conjunctivitis affects between one-fourth and one-half of infants born by the women who have *C. trachomatis* infection. The incubation phase typically lasts between 5 to 12 days after birth, although it can extend up to 6 weeks. Even though inclusion conjunctivitis affects the majority of babies, 10% to 20% of them progress to pneumonia. *C. trachomatis* infection acquired during pregnancy may last longer than two years in the nasopharynx, urogenital tract, or rectum (Chan et al. 2016).

E- Infant Pneumonia

10-20% of infants who contracted the mother's infection might suffer respiratory problems 2–12 weeks after birth, leading to pneumonia. Infants with the condition experience nasal blockage or discharge, severe tachypnea, a recognizable paroxysmal staccato cough, no temperature and eosinophilia. On radiographs, interstitial infiltrates and hyperinflation can be visible. An immunoglobulin M (IgM) antibody titer to *C. trachomatis* of 1:32 or greater was regarded as diagnostic in such newborn pneumonia. Systemic erythromycin is a good therapy for severe patients but oral erythromycin for 14 days is generally advised (Batteiger and Tan 2015).

Laboratory Diagnosis

Cytology, culture, direct detection of antigen or nucleic acid amplification test (NAAT), and serologic testing can all be used to detect *C. trachomatis* (Gaydos and Quinn 2005; Lefebvre 2014).

Treatment

Azithromycin, doxycycline, erythromycin, tetracycline, fluoroquinolones and other macrolide antibiotics are common drugs used to treat diseases that are caused by *C. trachomatis* (Kong et al. 2014).

Chlamydia pneumoniae

In 1965, a Taiwanese child's conjunctiva was the source of the initial isolation of the TWAR strain of *C. pneumoniae*. Because the inclusions formed in cell culture like those of *C. psittaci*, it was previously thought to represent a strain of psittacosis. The newly found strain was given the name "TWAR," an abbreviation for TW and AR, because the Taiwan isolate (TW-183) is serologically close to a pharyngeal isolate (AR-39) identified from a student in college from the United States **of** America (acute respiratory). There is just one recognized serotype of *C. pneumonia* (Pospisil and Canderle 2004).

General Characteristics

Due to the uniformity of the MOMP, all of the studied isolates of *C. pneumoniae* are immunologically identical, making it more homogeneity than both *C. trachomatis* and *C. psittaci*. The pear-shaped form of *C. pneumonia's* EB is one major variation between it and other *chlamydia* species (Carroll et al. 2016).

Pathogenesis

C. pneumoniae seems exclusively to be a human pathogen and did not determine in animals or birds as a carrier for disease. It is spread from individual to individual by nasal discharges via respiratory path. Of interest, *C. pneumoniae* infections are both endemic and epidemic. Regrettably, a few information were registered about the infection and the pathogen of *C. pneumoniae*, however it is analogues to *C. trachomatis* regarding for stimulation of inflammation that contributes to tissue damage (Hammerschlag et al. 2015).

Clinical Findings

Pneumonia, bronchitis, pharyngitis, sinusitis, and a flu-like disease have all been related to *C. pneumoniae*. It causes 5% to 10% of cases of community-acquired pneumonia. Young adults often have mild to moderate infections; the main characteristics of the clinical and laboratory differential diagnosis are infection with *Mycoplasma pneumonia* (Watson and Alp 2008).

There are multiple diseases of the upper and lower airways caused by *C. pneumoniae*. Pharyngitis is the most frequent disease to occur. Lower airway disease can develop together with sinusitis and middle ear infection. Elderly or people with impaired respiratory systems could get severe pneumonia. It should be noted that *C. pneumoniae* frequently causes asymptomatic infection which is undiagnosed, or the slightly symptomatic infections. Furthermore, there is a link between *C. pneumoniae* infection and the formation of asthmatic signs (Wolff et al. 2018).

Laboratory Diagnosis

The most sensitive test for diagnosing *C. pneumoniae* infections is the MIF test, which is used in laboratories to detect *C. pneumoniae* infections by cytology, serologic testing, and other means. By utilizing the proper reagents, the test is species-specific and may identify IgG or IgM antibodies. Assays known as NAATs are also used to identify *C. pneumoniae* nucleic acid sequences in clinical specimens. Additionally, pharyngeal swab samples should be placed in a *chlamydia* transport medium and kept at 4°C for cell culture. *C. pneumoniae* develops better in HL and HEp-2 cells than in HeLa 229 or McCoy cells (Conklin et al. 2013).

Treatment

Tetracycline, certain fluoroquinolones, and the macrolides all could destroy *C. pneumoniae*. The treatment of *C. pneumoniae* infectious diseases with doxycycline, azithromycin, clarithromycin, levofloxacin, or moxifloxacin appears to be highly beneficial for patients (Hammerschlag et al.2015).

Chlamydia psittaci

General Characteristics

The elementary and inclusion bodies of *C. psittaci*, as well as its sulfonamide resistance, distinguishes it apart from *C. trachomatis*. Although birds and domestic animals frequently get this chlamydial species, human infections are very rare (Zhang et al. 2022).

Pathogenesis

All bird species have an endemic pathogen called *C. psittaci*. Psittacine birds, such cockatiels, parrots, and parakeets, are a significant source of disease in humans. Employees at chicken slaughter and working facilities, veterinarians, veterinary technicians, laboratory staff, avian quarantine station staff, taxidermists, farmers, animal rehabilitators, and zoo staff are among the professions thought to be at the highest risk for psittacosis (Kalmar et al. 2014; Vorimore et al. 2015).

The birds might be asymptomatic or suffer diarrhea. The disease is spread among people by aerosol inhalation. The organisms are released into the alveoli, where some of them are later taken up by alveolar macrophages and transmitted to nearby lymph nodes. From there, they spread all over the body while developing inside the cells of reticuloendothelial system. Because human-to-human transmission is uncommon, hospital patients are not required to be isolated (Balsamo et al. 2017).

Clinical Findings

Typically, a 5 to 15-day incubation period precedes the onset of a disease (Schlossberg 2015). The initial phase might be sneaky or sudden. The infection can appear clinically in people in a variety of ways, from silent infection or a moderate flu-like disease to a systemic illness with extreme unusual pneumonia. The usual symptoms of symptomatic infection comprises of an unexpected beginning of headache, fever, chills, malaise and myalgia. Additional symptoms include non-productive coughing, trouble breathing, chest tightness, changes in mental state, and hepatosplenomegaly (Chaber et al. 2021).

Laboratory Diagnosis

Psittacosis is usually diagnosed using serologic techniques. Only laboratories with biosafety third stage biohazard containment facilities may safely cultivate *C. psittaci* due to the risks involved in working with the agents. State health agencies actively engage in physician consultations concerning potential instances (Van Lent et al. 2012; Wolff et al. 2018).

In individuals with suspected psittacosis infections, complement fixation and indirect micro-immuno-fluorescence have been employed to identify anti-*C. psittaci* antibodies. It is possible to attempt the isolation in cell cultures, but it's necessary to keep in mind that *C. psittaci* can infect lab animals. NAATs are not yet available for purchase. There have been tests using wide spectrum, multi-species pan-*Chlamydia* probes (Read et al. 2013; Balsamo et al. 2017).

Treatment

The preferred treatment for psittacosis is tetracycline. When it is not treated, it has a 20% mortality rate (Schlossberg 2015).

Conclusion

Chlamydia is microscopic organisms that proliferate in the cytoplasm of their host cells and also has distinctive biphasic developmental cycles. *Chlamydia* species that can cause human disease include *C. trachomatis*, *C. pneumoniae*, and *C. psittaci*. *C. trachomatis* is the cause of sexually transmitted diseases. A range of upper and lower respiratory infections are produced by the *C. pneumoniae*. Just contact with birds can transmit *C. psittaci*. Psittacosis might be moderate or obvious, but it has also been shown to cause severe pneumonia and sepsis, both of which have a significant fatality rate.

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12

Vibrio cholerae: Epidemiology, Surveillance and Occurrence in Iraq

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INTRODUCTION

Cholera is caused by the gram-negative bacteria *Vibrio (V.) cholerae*, which in turn causes diarrhea. *V. cholerae* occurs in a habitat of fresh and salt water and is endemic to hot countries such as Latin America, South of Asia, and some areas of Africa. (Baker-Austin et al. 2013; Ali et al. 2015). Approximately three to five million people are infected with cholera each year, resulting in about 140,000 fatalities, half of which are children younger than five years old (Ali et al. 2015). As the global climatic modification and rising temperatures, *V. cholerae* is spreading to a new areas, which is requiring the creation of an emergency research to better understand this bacterium (Vezzulli et al. 2016; Deeb et al. 2018). Most *V. cholerae* are "non-O1/O139" environmental isolates which may or may not cause gastroenteritis, but only strains classified as O1 or O139 can cause pandemic cholera (Baker-Austin et al. 2018). The genome of *V. cholerae* includes 2 circular DNA. Cholera toxin (CT), a protein that leads to serious and unique watery diarrhea (known as "rice-water stool"). The toxin is produced by coding genes present in CTXphi (CTXφ) which is a temperate bacteriophage. The Insertion of the viral gene into the DNA of a bacteria is necessary for the production of the toxin (Al-Obaidi 2006; Arif et al. 2010).

Due to poor sanitation and hygiene procedures and slow treatment and prevention as per inadequate healthcare system, cholera is endemic in many developing countries. A report by the Islamic Relief Project indicated 1,500 deaths in Iraq in 2015 due to Cholera outbreak. There has been a new outbreak of cholera in Iraq on 20th of June 2022. The Iraqi Ministry of Health recorded 13 cases, most of which have been found in the Sulaymaniyah governorate/Kurdistan region (Qamar et al. 2022a).

The Causative Agent

the most important characteristics of *V. cholerae* are; comma-shaped, Gram-negative and facultative anaerobic bacteria. The bacteria can survive in salt water environments and readily attach to the chitin of shells of shrimps, crabs, and other crustaceans. Cholera is a potentially fatal disease spread by eating raw or uncooked marine life (Tarsi and Pruzzo 1999).

The bacterium is supplemented with a flagellum at one end and numerous pili all over its exterior. Their metabolic processes involve; aerobic respiration and anaerobic fermentation. Cholera outbreaks usually caused by two different serotypes or serogroups: O1 and O139. (Ramamurthy et al. 2020).

Clinical Symptoms

Cholera is a highly virulent disease that can trigger severe cases of vomiting and watery diarrhea. An individual could develop symptoms 12 hours to 5 days after consuming contaminated water or food. Cholera is a disease that can kill both children and adults within hours if they are not treated (Azman et al. 2013). Most infected people with *V. cholera* are asymptomatic, but the bacteria can live in their stool between 1 to 10 days after infection to be sheded into the environment, where they can infect others. Most people who get sick have only mild to moderate symptoms, while a few get extremely sick with acute watery diarrhea and severe dehydration, shock and finally death If left without treatment (Sack et al. 2004; Legros 2018).

Pathogenesis

Cholera specificity is to the small intestine only and nowhere else in the body. *V. cholerae* possess one or more adhesion

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factors that permit them to stick to the micro-villi and allowing them to take up residence and grow in the human small intestine (which is normally relatively free of bacteria due to the effective clearance mechanisms of peristalsis and mucus secretion). It has been hypothesized that a number of toxin-co-regulated pili and hemagglutinins play an important role in adherence, but the underlying mechanism has not yet been understood. It's possible that several mechanisms may be involved. Vibrio motility may contribute to pathogenicity by allowing the organisms to destroy the mucus barrier. Proteases, mucinolytic and neuraminidase enzymes are also produced by the bacteria (Ojeda Rodriguez and Kahwaji 2022).

Pathogenic genes in *V. cholerae* express proteins that play a role in the bacteria's virulence either directly or indirectly. Outer membrane vesicles (OMVs) which is a protective membrane modifications is released by *V. cholera* and help the bacterium adaptation to the intestines of a host to evade the host's defenses, such as bile acids and antimicrobial peptides (Ramamurthy et al. 2020).

By entering the small intestine of a human host, the genes encoding virulence factors expression starts to produce toxin-co-regulated pilus (Tcp) and cholera toxin. Cholera toxin, which is made up of two different parts called CtxA and CtxB, binds to the ganglioside (asyalylated glycosphingolipid) GM1 on the cell membrane of intestinal epithelium. Once cholera toxin is bound, it is endocytosed and retrogradely transported to the endoplasmic reticulum (ER), where the toxin's subunits are released. CtxA subunit allosteric activation by ADP ribosylation factor 6 (ARF6) requires its release from the endoplasmic reticulum (ER) into the cytosol (Almagro-Moreno et al. 2015). Adenylyl cyclase is stimulated when the ARF6-bound, active CtxA subunit catalyzes the ADP ribosylation of a G protein-coupled receptor. Intense diarrhea is caused by an elevation in cellular cAMP levels, which in turn stimulates the protein kinase A (PKA)-dependent phosphorylation (P) of the cystic fibrosis transmembrane receptor (CFTR) (Baker-Austin et al. 2018; Chen et al. 2022).

Immunity To *V. cholerae*

Pathogenic *V. cholerae* strains are distinguished from non-pathogenic strains by their ability to produce cholera toxin (CT) and a toxin coregulated pilus (TCP) (Baek et al. 2020). Bacteria, intestinal epithelial cells and mucus are all floating within watery diarrhea. The mucus layer that normally covers intestinal epithelial cells performs numerous tasks, such as acting as an energetic protective barrier against flora and foreign microbes. This mucus gel layer is secreted by goblet cells which are the specialized cells that line mucosal tissue (Cornick et al. 2015). It is postulated that mucin manufacturing can be altered by 2 methods; either by microbial factors that modify the release and production of mucin, or by host factors that are produced by epithelial cells or immune cells in reaction to intestinal bacteria (Grondin et al. 2020). The effects of CT of the bacteria triggers the production of vast quantities of mucin through a cAMP-dependent mechanism, which contributes to the diarrhoea creation. It has been shown that *V. cholerae* can use its flagellum to invade mucus membranes, while non-motile Vibrios are either unable to colonize or are avirulent (Herath et al. 2020). Recent research has demonstrated that exposure to *V. cholerae* outer membrane vesicles (OMVs) can prime CD4+ T cells for an inflammatory T helper 2 (Th2) response and increase expression of the IL-17, IL-13 and IL-4 cytokines, all of which have been linked to an increase in mucin output (Melhem et al. 2021).

CD4+ T cells appear to be influenced by *V. cholerae* and CT in a way that promotes their differentiation to both Th1 and Th2 cell lineages, with a possible bias toward the latter. When T cells are activated by intracellular bacteria, they usually develop into the Th1 cell ancestry and fasten a cellular response, by the activation of phagocytes like macrophages and cytotoxic T cells (CD8+ T cells) rather than antibody production. A humoral immune response, include B cell activation and immunoglobulin generation, is triggered by excitation of Th2 cells in response to extracellular pathogens (Grondin et al. 2020).

In spite of the significant effort put into studying the immunology of cholera and the pathogenesis of *V. cholerae*, many un-answered questions persist. The most pressing issue is developing more effective cholera vaccines, as the existing one only protect 60% against the disease (Shaikh et al. 2020).

Diagnosis

Nevertheless, the criterion for a cholera diagnosis in the lab is the isolation and identification of *V. cholerae* serogroup O1 or O139 from a fecal sample through culture. The Cary Blair media and thiosulfate-citrate-bile salts agar (TCBS), are ideal for transporting isolation and identification of pathogenic bacteria in addition to serogrouping *V. cholerae* isolates by specific reagents. Rapid diagnostic tests available for purchase are helpful in times of epidemic but should not be used for routine diagnosis because they do not produce an isolate for antimicrobial susceptibility testing and subtyping (CDC 2022).

The standard for confirming a cholera diagnosis, is the isolation of pathogen from a stool or rectal swab culture. Culture should be on alkaline peptone water then streak on the media TCBS, or taurocholate- tellurite gelatin agar to grow the bacteria taken by the swabs. After initial culture in non-selective media, *V. cholerae* O1 and O139 detection must be confirmed by slide agglutination using specific monoclonal or polyclonal antibodies (Somboonwit et al. 2017; Falconer et al. 2022). However, the distinguishable darting motility of *V. cholerae*, which is inhibited by *V. cholerae* specific antibody, allows for rapid detection using dark field microscopy, which is only about 50% as sensitive as culture (Martinez et al. 2010). Rapid and low-cost tests are crucial for the diagnosis of cholera in both clinical and public health settings. Unfortunately, there are currently a few tests available, including the the Sensitive Membrane Antigen Rapid Test, co-agglutination test, Medicos and the Institute Pasteur and Crystal VC rapid dipsticks. The use of PCR assays is restricted, and they are only performed in specialized labs (Falconer et al. 2022).

Treatment

The cholera can be treated with simple medication. When given quickly, oral rehydration solution (ORS) can effectively treat the vast majority of patients. One sachet of the WHO/UNICEF recommended oral rehydration salts (ORS) is mixed with 1 L of purified water. On day one, moderately dehydrated adults may need as much as 6 L of ORS (Williams and Berkley 2018). Patients with diarrhea receive antibiotics to reduce the severity of their symptoms, the amount of rehydration fluids required, and the time spent excreting *V. cholerae* in their feces (Hsueh and Waters 2019). As long as patients are diagnosed and treated promptly, the case mortality rate can be kept well below 1%. In children younger than 5 years, zinc is an essential supportive therapy that not only shortens the length of diarrhoea but also protects against forthcoming episodes of severe watery diarrhoea due to other reasons. Encouragement of breastfeeding is also important (Lazzerini and Wanzira 2016). Patients are typically advised to take doxycycline first, while children and pregnant women should take azithromycin. In South Asia new strains of bacteria are appearing that are multiple drug resistance, and they are also resistant to trimethoprim-sulfamethoxazole, tetracycline and quinolones. Although the Hispaniola strain is multiple drug resistance , it can be treated with tetracycline and doxycycline (N'cho et al. 2022). To compete the MDR of *V. cholerae*, a non-traditional antimicrobial strategy should be used in the treatment and management of *V. cholerae* infection such as Nanoparticles (Das et al. 2018)**.** Nanotechnology has recently assumed greater significance in the biomedical and pharmaceutical sectors as an alternative antimicrobial plan (Desselberger 2000). When compared to chemical and physical synthesis methods, the biosynthesis of green nanoparticles using natural extracts of different organisms offers significant economic and environmental benefits (Samuel et al. 2022). It is hypothesized that their biocidal efficacy results from the close interactions with microbial membranes made possible by their small size (100 nm) and high surface-to-volume ratio (Morones et al. 2005; Allaker 2010). Another perk of inorganic antibacterial agents like metals and metal oxides is that they are more stable than organic compounds, which are more susceptible to degradation (Sawai 2003; Sondi and Salopek-Sondi 2004). Zinc oxide, in particular, has garnered interest as a potential antimicrobial agent among these metal oxides. ZnO prevents the attachment and endocytosis of enterotoxigenic *V. cholerae* (Sarwar et al. 2017).

In addition, ZnO's nanoparticle size makes it a potential bactericidal agent by inducing the generation of harmful oxygen radicals that can cause damage to DNA, cell membranes, and proteins (Salem et al. 2015).

Epidemiology, Risk Factors and Disease Burden

There are two types of cholera outbreaks: endemic and epidemic. In an endemic area, the disease is constantly present and has been there for at least three years (Ecarnot et al. 2021). In addition to endemic countries, cholera free countries can also undergo an epidemic outbreak. However, in endemic countries, cholera outbreaks can be seasonal or intermittent (Jutla et al. 2013). Having at least one cholera case confirmed in *cholerae* free country, means there is an outbreak in a country where cholera is not commonly found (Muzembo et al. 2022). Poor sanitation and water supply are major contributing factors in the spread of cholera. Places like slums on the outskirts of cities and refugee camps often fall into this category because they lack basic services like clean water and toilets. If cholera bacteria are already available or are introduced, the chance of spreading is increased due to the humanitarian crisis's effects, such as the destruction of water and sanitation facilities or the emigration of people to insufficient and overcrowded camps. Epidemics have never been linked to non-infected carcasses (Nsagha et al. 2015).

Prevention and Control

Ending Cholera: a global roadmap to 2030, is a strategic plan for cholera control, released in 2017 with the goal of reducing cholera disease mortalities to only 10% by 2030. It is predicted by scientists that about 21,000 to 143,000 individuals die yearly due to cholera infections around the world (GTFCC 2022). Cholera outbreaks can be contained and prevented with the help of oral cholera vaccines in affected area where this disease is common. Cholera continues to be a major health concern around the world and is a reflection of social inequality and stagnation (Kanungo et al. 2022).

Cholera control requires multiple strategies working in tandem. Oral cholera vaccines and therapies are used alongside other measures such as surveillance and WASH (water, sanitation, and hygiene) initiatives and community outreach (Kanungo et al. 2022).

Surveillance

Surveillance for cholera must be a part of a larger system for monitoring the spread of disease, for international collaboration (Ngwa et al. 2016). Cholera can be detected more easily with the help of rapid diagnostic tests (RDTs), where the presence of even one positive sample will authorize a cholera warning (Chowdhury et al. 2021). In order to confirm the results, the specimens are sent to a lab where they can be cultured or tested with polymerase chain reaction. The effectiveness of a surveillance system and the preparation of control legislation depend critically on the local capacity to detect (diagnose) and monitor (collect, compile, and analyze data) cholera occurrence. In order to better identify and react to cholera outbreaks, it is recommended that countries at risk improve their disease surveillance and national readiness. When deciding whether or not to issue an official alert, public health officials must always compare the circumstances surrounding a cholera outbreak to the standards listed in the International Health Regulations (Ganesan et al. 2020).

Water and Sanitation Interventions

To effectively combat cholera over the long period of time, there is need of an economical investment and ensurance that everyone has access to clean water and proper sanitation. Implementing long-term sustainable WASH solutions to ensure the use of safe water, basic sanitation, and good hygiene standards in cholera zones is the primary action promoting environmental conditions. In addition to preventing cholera, these sorts of intervention strategies help in the fight against poverty, malnutrition, and the lack of education. Water, Sanitation, and Hygiene (WASH) measures to combat cholera are aligned with Sustainable Development Goals (SDG) targets (Challa et al. 2022).

Community Engagement

To be "community engaged" is to have people and communities take part in planning and executing projects. The adaptation of preventative hygiene measures, such as hand washing with soap, safe food preparation and storage, and safe disposal of children's stool, is greatly facilitated by local cultural practices and beliefs, as are funeral rituals for cholera victims that reduce the likelihood of the disease spreading to mourners (WHO 2020).

Oral Cholera Vaccines

Dukoral®, ShancholTM, and Euvichol-Plus® are the three currently available WHO-approved oral cholera vaccines (OCV). All three vaccines require 2 doses for full protection (WHO 2022a).

WHO Position Paper on Vaccines against Cholera in 2017 stated that "oral cholera vaccines must be used in locations with endemic cholera, in humanitarian disasters with a greater risk of cholera, and during cholera outbreaks; always in combination with other cholera prevention and control strategies; vaccination should not hinder the requirement of other urgent healthcare systems to govern or inhibit cholera outbreaks" (WHO 2022a).

Non-Cholera Infections

The most common types of human disease caused by Vibrio bacteria are cholera and non-cholera infections (Baker-Austin et al. 2018).

Non-cholera Vibrio spp. other than *V. cholerae*, such as *V. parahaemolyticus* and *V. vulnificus*, cause a group of infections with varying clinical features based on the bacterium species, route of infection, and host susceptibility. Mild gastroenteritis or primary septicemia can result from ingesting non-cholera bacteria, while exposing skin wounds to contaminated water can lead to a wound infection and, potentially, secondary septicemia. Non-cholera Vibrio spp. occur naturally in sea water and seafood, and prefer environments with a medium to high salinity. These bacteria thrive in aquatic and marine settings (Baker-Austin et al. 2018).

V. cholerae in Veterinary Medicine

One of the very important zoonotic bacterium is *V. cholerae* which has been found to infect marine mammals in a variety of environments, including fresh and salt water (Chen et al. 2022). Dogs can become infected with cholera if they consume a large quantity of food or water contaminated with *V. cholerae*. Epidemics in bison, dogs and cattle have already been observed (CFSPH 2006).

Though restricted discharge standards have been constructed for aquatic wastewater because of the industry's rapid growth, lax monitoring inevitably leads to environmental pollution and the subsequent spread of pathogens (Chen et al. 2022).

Bacterial infections were a major issue in the onset of intensive Atlantic salmon farming, specifically cold water vibriosis (*V. salmonicida*, *V. anguillarum*), and furunculosis (Aeromonas salmonicida ssp. salmonicida). The use of antibiotics to treat these diseases was only moderately successful at first, but vaccination eventually proved to be the most effective method for eradicating them (Novotny et al. 2004).

External and internal normal flora of fish typically include vibrios, which are common, facultative bacteria. Salmonids and other fish and aquatic animals kept in marine and brackish environments are susceptible to vibriosis. Vibriosis, also known as saltwater furunculosis, has been linked to MAS and furunculosis. *V. anguillarum* is the most prevalent pathogenic species in salmonids (Webster and Lim 2001).

However, fish are the vector for the *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*, all of which are found in the marine ecosystem (Novotny et al. 2004).

V. cholerae in Iraq

Cholera epidemics could break out in Iraq because the country is on the path of pilgrims take to reach Mecca and houses several important religious sites. During the cholera outbreak of 1820, many people in Basra, Iraq, died. In Baghdad, the disease had a similar impact. After that, the disease vanished from Iraq altogether, only to re - emerge in August 1966 as part of the seventh pandemic wave. Since then, the occasional outbreaks have continued in Iraq. After the Gulf War in 1991, cholera spread to every governorate in Iraq. The rural areas suffered the most during the months of April through November, when the danger level was the highest (Khwaif et al. 2010).

Baghdad city records represented one-third of all reported cases in Iraq during the 1991 epidemic, but for significantly lower percentage of the total cases infected during the years between the two epidemics of 1991 and 1998. In 1999, however, Baghdad once again contributed approximately one third of all reported cases. From 1999 to 2005, only 35 cases were reported and in the three years prior to 2007, there were no September-December cholera recorded cases in Baghdad. In 2007, however, cholera broke out across Iraq (Al-Obaidi 2006 Jameel et al. 2016).

There were 4667 confirmed cases of cholera in Iraq in 2007, with 136 (2.9% of the total) being recorded in the capital city of Baghdad. In Baghdad, the cases were reported in the period from September to December. Five asymptomatic cholera cases were found to be carriers (0.5%). There were three cholera deaths in Baghdad, to comprise a case fatality rate (CFR) of 2.2% (Khwaif et al. 2010).

The frequency of cholera outbreaks in Iraq appears to repeat every four to five years. After the peak in 1998–1999, there were four more peaks in 2003, 2007, 2012, and 2015; the most recent epidemic impacted people of all ages and had a case count of around 3000. This long-term pattern may be attributable to the period of time needed for the microorganism to reproduce and reorganize itself in a large population, or to the buildup of a vulnerable cluster prior to the outbreak of an epidemic. Potential contributors to these pandemics include a lack of public knowledge about the diseases' transmission mechanisms, a dearth of medical professionals, and the destabilization of health infrastructure (including the availability of clean water) (Hussain and Lafta 2019).

Over the recent years, WHO has received a disproportionately large number of reports of cholera. As of March, 2020, there had been reported cases in 24 countries comprising of 323 369 cases, with 857 deaths. Many cases are not recorded due to gaps in surveillance systems and fear of impact on trade and tourism, which accounts for the difference between these statistics and the estimated burden of the disease (WHO 2022b).

On June , 2022, the Central Public Health Laboratory (CPHL) confirmed 13 cases of cholera in Sulaymaniyah, Kirkuk, and Muthanna (WHO 2022d).

As of August, 2022, 865 cases of cholera had been confirmed in Iraq, resulting in 4 deaths. Thi -Qar, Baghdad-Rasafa, and Kirkuk are the governorates with the highest numbers of cases and fatalities: 55, 224 and 480 cases respectively. The Early Warning, Alert and Response Network (EWARN) system has also received reports of acute diarrhea cases in camps (WHO 2022c).

If the case fatality rate (CFR) for cholera is less than 1%, as recommended by the World Health Organization (proper cholera case management should keep CFR below 1%), then the case management is successful. Many water sources in the Sulaymaniyah governorate have been treated, and the water trucking industry has been enlisted to help to get clean water to the people who need it. Cholera cases decreased in the Sulaymaniyah province as a result of this and effective crisis intervention (WHO 2022c) .

Social and economic instability in Iraq has persisted for decades, creating problems with access to healthcare, labor availability, and the availability of necessary supplies. From 2014 to 2017, the Islamic State of Iraq and the Levant (ISIL) employed a strategy of aggression in which it deliberately destroyed water organization and delivery systems in order to flood cities, disturb agriculture, and withdraw residents of access to Water, Sanitation, and Hygiene (WASH) resources. (Qamar et al. 2022b).

Iraq is particularly susceptible to waterborne and infectious diseases due to its site lengthways the Tigris and Euphrates, whose polluted water provide the greater part of the country's water needs, and its proximity to adjacent countries that experience frequent disease outbreaks. The result was a devastating cholera epidemic in 2015, with the maximum number of informed cases appearing in October after unusually heavy precipitation (WHO 2022d). With the importance of these risk factors for Cholera clearly established, strategies to aid the Iraqi public, such as early detection, management, and treatment, become crucial (Chen et al. 2022). As of the 20th of June, 2022, the Iraqi Ministry of Health is dealing with 13 cases of Cholera, most of which have been reported in the Sulaymaniyah province of the Kurdistan region. The recent shipment of medical supplies to the Kurdistan region attests to the United Nations continuing to cooperate with the Ministry of Health of Iraq to improve healthcare outcomes during the Cholera outbreak as of June 2022 (WHO 2022d) Local health officials are committed to preventing the disease's spread and have been conducting awareness campaigns, monitoring and treating water sources, and conducting routine food inspections to demonstrate their resolve. The local health authorities' encouraging response is consistent with a U.N. initiative to encourage governments to be more pro-active in providing basic necessities to the poor and vulnerable (Qamar et al. 2022a).

Conclusion

Infection with the bacterium *V. cholerae*, (Cholera) continues to be a major health problem in many parts of the world. After experiencing an outbreak of cholera in 2015, Iraq is still fighting with the disease today. Inadequate infrastructure, ineffective healthcare system, inadequate sanitation and hygiene, malnutrition, and a military conflict state; all play a role in the spread of cholera. Rehydration treatments, antidiarrheals, vaccinations, food, and monetary aid are still being distributed as part of massive-scale relief efforts. Long-term health gains can only be achieved through the application of education campaigns and the development of better facilities and public healthcare facilities. More information and study are needed to devise methods for controlling Cholera's spread and bringing down its worldwide prevalence.

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Campylobacteriosis

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INTRODUCTION

Campylobacteriosis has been identified as an important food-borne zoonotic disease around the world, and it is caused by members of the genus *Campylobacter* which is ubiquitous bacteria (Iannino et al. 2019). The *Campylobacter* genus belongs to the family Campylobacteraceae and the genus consists of 39 species with 16 subspecies (Facciolà et al. 2017; Hlashwayo et al. 2020). The majority of infections in humans are caused by the thermophilic species *Campylobacter (C.) jejuni*, followed by *C. coli*, *C. lari*, and *C. upsaliensis*. (Leonard et al. 2011; Authority et al. 2013). The pathogen exists as normal flora in the intestinal tracts of many animal and bird species without causing any serious disease symptoms (Takamiya et al. 2011). There are several ways of disease transmission to humans, including consumption of undercooked meat or contaminated meat especially poultry, dairy product, contaminated water, as well as contact with diseased animals or feces (Zhang et al. 2018). In the modern society, *Campylobacter* has become the most common bacterial cause of human foodborne gastroenteritis worldwide (Acheson and Allos 2001). Along with gastrointestinal infections, it also causes various human systemic infections, including bloodstream infections, endocarditis, septic thrombophlebitis, pneumonia, neonatal sepsis, acute appendicitis and acute colitis of inflammatory bowel disease (Morishita et al. 2013; Alnimr 2014; Lagler et al. 2016). Other post-infection complications include severe demyelinating neuropathy, Guillain-Barre syndrome (GBS) and Miller-Fisher syndrome (MFS) (Scallan et al. 2015; Skarp 2016). *Campylobacter* infection are often common in particular age group, such as children under four years of age and people over 75 years (Levesque et al. 2013). Patients with hemoglobinopathy, inflammatory bowel illness and individual with impaired immune systems are at higher risk of getting *Campylobacter* infection (Kennedy et al. 2004). Underdiagnoses and underreporting of the disease have been linked to difficulties in isolating and growing *Campylobacter* species, their high cost and the lack of a legal requirement for its monitoring in foods. As a result, information about outbreaks or the contamination of animal products does not accurately reflect the state of the nation (Mendonca et al. 2015).

Most of the time, *Campylobacter* infection is self-limiting and requires supportive therapy. However, antimicrobials may be needed in patients with severe, persistent and extraintestinal campylobacteriosis and in immune-compromised patients (Kaakoush et al. 2015). The presence of this pathogen in animal products and other foods that might contain these agents can pose a risk to human health (Facciolà et al. 2017).

History

Since 1909, it has been known that the pathogenic bacterium can result in animal abortion, especially in bovines and ovines. Two veterinary surgeons, McFadyean and Stockman (1913), studied epizootic abortion in sheep and discovered an unidentified bacterium that was frequently isolated from aborted fetuses and that resembles a *Vibrio (V.) fetus*. Initially, the classification of bacteria was based on morphology. Vincent (1947) isolated *V. fetus* from three pregnant women who were taken to a hospital with an unidentified fever. On inspection of the placenta, large necrotic and inflammatory regions were visible. Two of the three women had abortions, and the illness lasted for about four weeks. Sebald and Véron (1963) renamed this organism as *Campylobacter*. Only the blood of bacteremic individuals could be used for diagnosis of infection. Dekeyser et al. (1972) in collaboration with Butzler and his group at the St. Peter University Hospital, successfully isolated *Campylobacter* from feces. In 1980, Penner and Hennessy introduced serotyping methods that still serve as the foundation for strain typing today with the help of genotyping, phage typing, and biotyping. Until the middle of the 1980s, *C. jejuni* was known to be the most frequent cause of bacterial enterocolitis in humans. These days, *Campylobacter* has become a serious issue for both industrialized and developing nations' public health (Butzler 2004).

Etiology

Campylobacter species is gram negative, spiral rods or spirally curled in shape, ranging in size from 0.2–0.8 µm broad and 0.5–6.0 µm long and even cells can change to coccoid or spherical forms in reaction to stress or harmful conditions (Shane 1992; Kassem et al. 2017). They occur as

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normal inhabitants in the gastrointestinal tracts of a number of domestic animals and birds such as commercial broiler chickens (Aydin et al. 2001; Zimmer et al. 2003). There are at least 30 recognized species and subspecies in the genus *Campylobacter* (On 2013). They belong to the Campylobacteraceae family, a broad but phylogenetically separate group of Gram-negative bacteria that is classified in the Proteobacteria's epsilon division (Lastovica et al. 2014). Additionally, the family Campylobacteracea includes the genera *Arcobacter* and *Sulfurospirillum* (Fitzgerald and Nachamkin 2015). Thermophilic *Campylobacter* spp. (*C. jejuni*, *C. lari* and *C. coli*,) are recognized as being clinically significant as these are the primary causal agents of human campylobacteriosis (Hermans et al. 2012). All species lack spores and have a single bipolar flagellum that mediates a distinctive corkscrew-like or darting movement that can be seen in dark fields of illumination (Byrd et al. 2007). They require a microaerobic environment for optimal growth and are slow-growing, picky organisms that grow best at 37°C to 42°C on artificial media (Lastovica et al. 2014). Since the bacteria are often unable to ferment carbohydrates, the breakdown of amino acids serves as the main source of energy for the organism, and they are oxidase and catalase positive, indole negative and reduce selenite (Simon 1994; Kelly 2001).

Incidence and Distribution

The epidemiology of campylobacteriosis is considered complex. The rate of accurate incidences of *Campylobacter* epidemic is largely unknown. This is because campylobacteriosis outbreaks are rarely reported, and there are difficulties with diagnosis and variations in surveillance (Hansson et al. 2018). Until now, foodborne campylobactereiosis has been seen to increase in the health issue in both undeveloped and developing countries. Each year, there are more positive cases in North America, Europe, Australia, and New Zealand (Man 2011; Kaakoush et al. 2015). The frequency of *Campylobacter* infection is also high throughout Asia, Africa, and the Middle East despite the absence of adequate epidemiological data (Butzler 2004). Some studies report that in developed countries, the prevalence of *Campylobacter* causes gastroenteritis with a frequency of two to seven times higher than *Escherichia coli* O157:H7, *Shigella spp*., or even *Salmonella spp*. (Acheson and Allos 2001; Authority et al. 2014). Although most *Campylobacter* outbreaks are from waterborne or foodborne illness and most of these outbreaks are usually from animals (Frost et al. 2002), At least 80% of foodborne *Campylobacter* outbreaks were carried on by poultry products (Hsieh and Sulaiman 2018). Despite the fact that most *Campylobacter* occurrences in low-income countries originate from natural sources like rivers and streams where large number of people depend on these water bodies as their main source of drinking water (Clark et al. 2003; Platts-Mills and Kosek 2014). In addition, high-income nations including Norway (Jakopanec et al. 2008), Finland (Kuusi et al. 2004), New Zealand (Bartholomew et al. 2014), Canada (Clark et al. 2003), and Denmark have indicated that water supplies have been linked to *Campylobacter* epidemics (Kuhn et al. 2017). There was seasonal variation in the prevalence of *Campylobacter*, the number increases to its peak in warm weather; while with dropping temperatures, the numbers decrease (Hermans et al. 2012; Sahin et al. 2015). Although the specific cause of this seasonal change is undetermined, it is hypothesized that a rise in fly population and fly-mediated transmission increase the number of *Campylobacter* in warm months (Hald et al. 2004).

Transmission Routes of Campylobacter Infection

Most of the animals, including poultry, cattle, sheep, dogs, cats, ostriches, pigs, and European blackbirds carry several *Campylobacter* species in their intestinal tracts (Dearlove et al. 2016). These animals' feces contain these bacteria, that are spread into the surrounding (Goni et al. 2017). Under typical ambient conditions, *Campylobacters* typically cannot proliferate in feed, litter, or water because they are sensitive to oxygen and temperature. (Sahin et al. 2002; Newell and Fearnley 2003). Contaminated litter by *Campylobacter* may contribute to the persistence of the bacteria in the farm environments (Line 2002; Sahin et al. 2002). It can be spread mechanically by rodents and insects like houseflies (Sahin et al. 2015). Movement of people and equipment between farms may spread *Campylobacter*, which has been implicated in several studies as a potential risk factor (Hansson et al. 2007; Sahin et al. 2015).

Consumption of contaminated or undercooked meat (particularly poultry), contaminated raw milk, and other handling of various products of animal origin are the most typical ways that *Campylobacter* spp. spread to humans (Bronowski et al. 2014; Kaakoush et al. 2015). Additionally, direct interaction between people and animals, such as pets, was reported as a possible route of infection transmission (Westermarck 2016), and people who work with cattle are at a significant risk of contracting *Campylobacter* because these animals are frequently colonized by it (Hansson et al. 2018). Furthermore, drinking water has been implicated as a major reservoir for the existence of *Campylobacter*; the most typical *Campylobacter* species found in surface water is *C. jejuni* (Hokajärvi et al. 2013). Finally, raw vegetables were identified to be the second major risk factor after consuming contaminated chicken meat; it can become contaminated with *Campylobacter* directly at farms, or during transport, and processing in food manufacturers (Kwan et al. 2008; Bronowski et al. 2014). Finally, transmission of campylobacteriosis can occur between humans either through fecal-oral transmission or, in males, by homosexual contact (Kuhn et al. 2021). Congenital transmission has been described but is a rare neonatal issue (Shane 2019). Fig. 1 presents a graphic overview of the transmission of *Campylobacter* in animals and man.

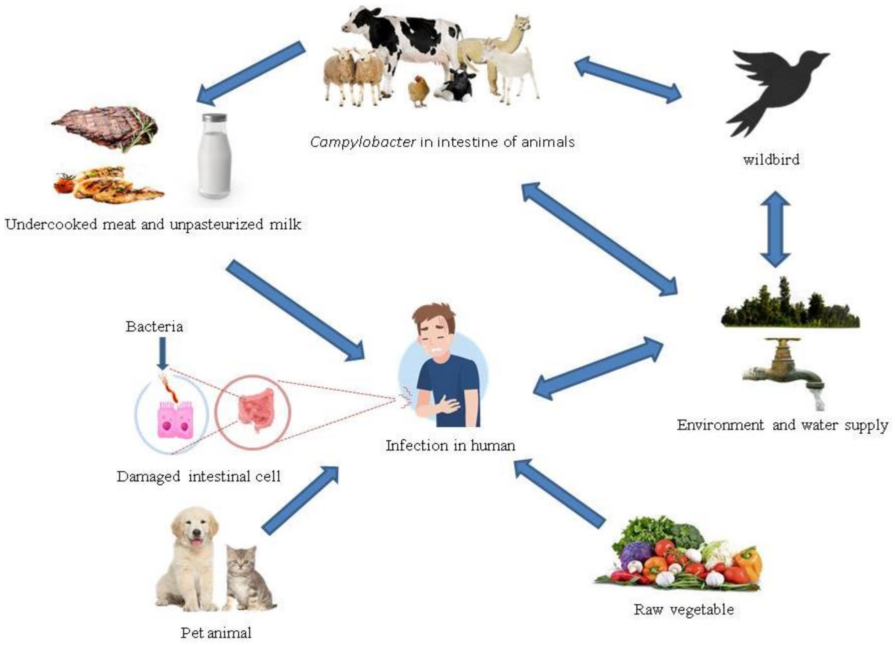
Campylobacter in intestine of animals
wildbird
Undercooked meat and unpasteurized milk
Bacteria
Infection in human
Environment and water supply
Damaged intestinal cell
Pet animal
Raw vegetable

Fig. 1: Overview of pathways of *Campylobacter* transmission in both Human and animals.

Infections in Animals

Clinical Sings

Many different animal species can carry *Campylobacter* spp. asymptomatically (Komba et al. 2013). According to certain research, experimentally inoculating young chickens and turkey poults with *Campylobacter* might result in clinical illnesses such bloody or mucoid diarrhea, weight loss, or even mortality (Lam et al. 1992; Humphrey et al. 2014; Zhang and Sahin 2020). The severity of enterocolitis that results from infection depends on both the pathogenicity of the strain and the host's immune system (Sanyal et al. 1984; Soerjadi-Liem 1984). Infection by *Campylobacter* in dogs and cats presents as acute enterocolitis and is characterized by mild to watery diarrhea, dehydration, lethargy, anorexia, vomiting, pyrexia, and abdominal pain (Marks et al. 2011; Sykes and Mark 2013). Cattle, sheep, and swine are also colonized by *Campylobacter* spp., and clinical symptoms in young animals could be more severe than those in adults (Mendonca et al. 2015). *C. jejuni* can cause bovine mastitis and enteritis in calves, whereas *C. fetus* causes abortion and infertility in cattle (Stanley and Jones 2003). Hamsters, neonatal mice, rabbits, and ferrets are also susceptible to intestinal colonization with *C. jejuni*, which may result in persistent diarrhea (Bell and Manning 1991).

Lesions

Enterocolitis with varying degrees of severity is the hallmark of mammalian campylobacteriosis (Shane and Montrose 1985). Gross lesions include catarrhal to severe hemorrhagic enteritis. In newly hatched chicks, there is an accumulation of fluid, gas, or excess mucus, as well as intestinal distention and watery/foamy material in the ceca is apparent (Sanyal et al. 1984). Experimental research revealed that a novel *Campylobacter* species, *C. hepaticus*, which causes the spotted liver disease in chickens, is known for causing widespread, 1-2 mm grey-white lesions in the liver (Crawshaw et al. 2015). Microscopic findings include villous atrophy, mononuclear infiltration of the submucosa, and a mild edema of the lamina propria and submucosa of the intestines, mostly in ceca (Stern et al. 1988). In sheep, gross fetal lesions include fibrinous peritonitis, hepatomegaly, perforated liver with internal bleeding, and many dispersed, round-to-targetoid areas of hepatic necrosis between 1 mm and 2 cm in diameter (Jubb et al. 2012). In cattle, abortion can occur at any gestational age, with lesions characterized by endometritis, placentitis, fetal serositis, hepatitis, and pneumonia (Michi et al. 2016).

Diagnosis

The diagnosis is made using cultural isolation and detection of the causative agent, biochemical characterization, recent molecular techniques and tools (Sails et al. 2003). In domestic animals and wild species (crows, mice, and chimpanzees), samples were obtained from feces (rectal samples if possible). In the case of avian species, samples were taken using cloacal swabs, poultry house droppings, or the caecal contents of dead birds (Komba et al. 2013). After a pre-enrichment in a liquid medium, plate positive broth on selective media (blood-based and charcoal-based), serve to remove toxic oxygen derivatives (Hsieh and Sulaiman 2018). Many media have been used for culturing *Campylobacter* species. Under laboratory circumstances, the bacteria are fastidious and slow-growing, needing a microaerobic environment (5% O_2, 10% CO_2, 85% N_2) at a higher temperature (42°C), with no growth being seen at temperatures below 31°C (Sahin et al. 2003). The most popular diagnostic methods for finding and identifying *Campylobacter* spp. in most laboratories are the enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) (Lilja and Hänninen 2001).

Treatment

Fluid replacement and antispasmodics are typically sufficient. In the case of diarrhea, pets and other domestic animals should receive symptomatic treatment. In case of pyrexia or prolonged and serious circumstances, antibiotic medication may be necessary (George 1994). In cats and dogs, erythromycin (10 mg/kg in cats, 10–15 mg/kg in dogs orally every eight hours) or azithromycin (5–10 mg/kg in dogs and cats orally every twenty-four hours for five to twenty-one days) have been suggested as treatments (Marks et al. 2011). Cefoxitin, erythromycin, or enrofloxacin have all been effective treatments for dogs with cholangiohepatitis/cholecystitis and bacteremia associated with *C. jejuni* (Sykes and Mark 2013).

Prevention

Strict biosecurity measures are the sole action that has been shown to be successful in preventing the entry of *Campylobacter* spp. to farms and herds (Newell and Fearnley 2003). These include: cleaning and sanitizing the entire house, including every piece of equipment between successive cycles of livestock, which will prevent horizontal transmission (George 1994). Programs to control insect (e.g., flies and cockroaches) will reduce the probability of infection (Hald et al. 2007). A set of management established to reduce the entry of unauthorized people, birds, rodents, or other animals to farms and herds (Facciolà et al. 2017). There should be proper water purification (chlorination) as it prevents the introduction of viable *Campylobacter* spp. (Guerin et al. 2007). Besides biosecurity, there is various ways used on farms for control of *Campylobacter* colonization, such as immunization, feed additives, bacteriocins, bacteriophages, and competitive exclusion (Johnson et al. 2017).

Infection in Human

Clinical Signs

The foremost symptom in foodborne campylobacteriosis is gastrointestinal illness, which manifests as an acute diarrheal disease with clinical symptoms, such as abdominal pain and fever. The diarrheal disease is usual 12-24 hours before the onset of clinical symptoms that presents a period of myalgia, pyrexia, and malaise (Mendonca et al. 2015). The symptoms are not distinct enough for the doctor to distinguish them from other bacterial infections induced on by food poisoning (Edward and David 2004). The incubation period may range from 1 to 5 days, reaching up to 10 days, based on the amount of infection and the strain of organism (George 1994; García and Cravioto 2007). Although the condition is typically self-limited and symptoms may disappear in a week, the consequences of dehydration, hepatitis, and neurological abnormalities may be seen (Akitoye et al. 2002). In addition to gastroenteritis, *Campylobacter* infections also result in reactive arthritis, irritable bowel syndrome, Guillain-Barré Syndrome (GBS), Miller Fisher Syndrome, bacteremia, septicemia, meningitis, cardiovascular complications, and abscesses (Fitzgerald and Nachamkin 2015; Kaakoush et al. 2015). Death as a result of campylobacteriosis is uncommon, but it can occur when the condition is widespread in people who are immunocompromised or in old or very young patients (Mendonca et al. 2015).

Lesions

The infection is most commonly occurred in ileum, jejunum, and colon. These lesions might include mild hyperemia, congestion, edema and the hemorrhages that ultimately leads to ulcerative and necrotic changes (George 1994). Histologically, edematous and bloody Jejunal, ileal, and colonic mucosa are present, and enlarged mesenteric lymph nodes may be seen with lymphocytic infiltration in acute cases, but polymorphonuclear leukocytes and eosinophils may accompany ulceration and abcessation (Blaser et al. 1980). Microscopic analysis of rectal biopsy samples from people who had severe *C. jejuni* enteritis revealed a mixed collection of inflammatory cells in the lamina propria, mucosal epithelial ulceration, loss of mucus, and crypt abscess in the epithelial glands (Cover and Blaser 1989).

Diagnosis

Fecal samples have been identified as preferred specimens for the detection of *Campylobacter* spp. in people who have digestive problems (Senok and Botta 2009). Isolation of *Campylobacter* spp. from the blood is confirmatory. Serological techniques have been established for the direct detection of *Campylobacter* spp. based on antigen-antibody interaction (Regnath and Ignatius 2014). For the molecular subtyping of *Campylobacter* isolates, numerous DNA-based techniques have been created. These include polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE), Multilocus sequence typing (MLST), random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (Fitzgerald et al. 2001).

Treatment

Many cases of campylobacteriosis require only supportive therapy. Sometimes antibiotics are given, especially when the symptoms are severe or prolonged and in immunocompromised patients (Same and Tamma 2018). Erythromycin, clarithromycin, and azithromycin are the antimicrobial agents of choice (Mendonca et al. 2015). Antibiotic-resistant diseases, particularly those resistant to fluoroquinolones, are on the rise as a result of the overuse of antibiotic in both human and animal populations (Iovine 2013). The use of a probiotic (Lactobacillus acidophilus) along with a prebiotic (fructooligosaccharides, lactosucrose) showed positive effects on the balance of intestinal microflora, as well as an improvement in recovery from infection and immune status during the investigation of alternative antibiotics sources for the treatment of campylobacteriosis (Rastall 2004).

Prevention

Foodborne campylobacteriosis can be minimized by taking the proper precautions while handling and preparing meals from an animal origin (Butzler 2004). It's important to properly cook raw meat and poultry because *Campylobacter* is sensitive to heat and cooking the food at 70 °C will reduce the chance of getting an infection (Facciolà et al. 2017). When preparing food in the kitchen, wash hands with warm water and soap, keep work area, kitchen sink, cutting boards and tools clean (Biabana 2004), and animal products and other foods should be prepared using different cutting boards and cutlery. Untreated water and unpasteurized milk products should not be consumed (FDA 2012). Diseases prevalence can be decreased by keeping appropriate refrigeration from the time of processing chicken and red meat to the moment of preparation (George 1994). Children, pregnant women, and immunocompromised people should be kept away from sick animals and animal feces (Campagnolo et al. 2018). Those who have campylobacteriosis should practice good hygiene to avoid the illness from spreading (Hemsworth and Pizer 2006).

Conclusion

Campylobacter enteritis in humans has become increasingly well-known during the past three decades, with current isolation rates exceeding those of *Salmonella* and *Shigella* spp.; the two common gastrointestinal infections. There is a possible relationship between *Campylobacter*-related infection in both humans and domestic animals. Strategic plans for campylobacteriosis prevention and control are also essential. Veterinary and medical professionals should need to work together in order to reduce the rate of infection transmission to human from animal sources.

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Bovine Anaplasmosis: Diagnosis, Treatment and Control Strategies

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INTRODUCTION

Anaplasmosis is an infectious disease that affects bovines when the pathogen, *Anaplasma (A.) marginale*, is transmitted from a carrier animal to a healthy (free) one (Sarli et al. 2021). Clinical presentation is characterized by fever, anemia, a reduction in food intake and, therefore, decrease in meat and milk production (Khan et al. 2022). However, clinical signs are not always evident but, in acute phase, adult cattle may be severely affected causing a high mortality rate while recovered cattle as well as infected calves become carriers for life and a source of distribution, presenting rickettsemia cycles every 6 to 8 weeks (French et al. 1998). Transmission is made by ticks, flies, horseflies and fomites. This is a crucial point as the disease is one of the most common and important vector-borne diseases in bovine production (Amaro-Estrada et al. 2020; Bautista-Garfias et al. 2021). Early diagnosis allows a better control and reduction of economic losses (Railey and Marsh, 2021). Even though there is not a universal vaccine commercially available, but there are several approaches in curse around the world to develop effective immunogens (Salinas-Estrella et al. 2022b) and many work still needs to do in order to generate better control strategies that contribute to improve bovine, human and environmental health.

Etiological Agent

Bovine anaplasmosis is caused by *A. marginale*, which is a gram negative intracellular obligate pathogen belonging to the order *Rickettsiales* which infects bovine erythrocytes (Kocan et al. 2010). It was first described by Sir Arthur Theiler (1910a,b) as “marginal points” in erythrocytes, therefore given the name “*marginale*” based on its location (Theiler 1908, 1910a, 1910b). Previously, it was believed that *A. marginale* was some developing state of *Babesia* spp. but later Theiler proved, by isolation and replication of the pathogen, that it was an entirely different microorganism (Theiler 1908, 1911).

For a while, any intracellular bacteria were considered among the term rickettsiae, despite any other characteristic; later, classification based on phenotypical, morphological, ecological, epidemiological and clinical characteristics followed (Drancourt and Raoult 1994) and then some molecular studies brought more clarity over this matter. A few years ago, taxonomic analysis based on the information of several molecular markers, antigenic characteristics, as well as pathogeny, helped to confirm and define its classification as member of one of the two families that belong to Rickettsiales order: Rickettssiae and Anaplasmataceae (Dumler et al. 2001). Members of the second family include the genus *Anaplasma*, *Ehrlichia*, *Wolbachia* and *Neorickettsia*, several species of *Anaplasma* genus that infect animals (*A. marginale*, *A. marginale* subspecie *centrale*, *A. ovis*, *A. bovis*, *A. platys*) and a specie that may infect humans as well as several mammals, (*A. phagocytophilum* which was formerly known as *Ehrlichia phagocytophila*, *E. equi* and the human granulocytic ehrlichiosis (HGE) agent) (Drancourt and Raoult 1994; Dumler et al. 2001).

Bovine anaplasmosis is produced when the microorganism enters the circulatory system and replicates within the erythrocytes. Genetic diversity (de la Fuente et al. 2005, 2007; Rodríguez et al. 2009; Castañeda-Ortiz et al. 2015) may influence the severity of the signs producing an infection that can be mild to severe according to the immune status of the animals and the virulence of the strain (García-Ortíz et al. 2000; Rodríguez-Camarillo et al. 2008; Rodríguez et al. 2009; Kocan et al. 2010).

Epidemiology

A. marginale is commonly found in tropical and subtropical regions around the world, which have the perfect conditions for developmental cycle of biological and mechanical vectors of the pathogen but, due to climate change, it can also be found on template regions because different species of vectors are prevalent in that climatic region (Kocan et al. 1992; Kocan et al. 2004). Prevalence of this disease has been

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reported in several countries of all continents, either by serologic or molecular analysis (Bautista-Garfias et al. 2021). The presence of the pathogen is associated with the presence of ticks –the biological vector– or flies –one of the mechanical vectors– (Scoles et al. 2005). Approximately 30 tick species have been reported as vector of bovine anaplasmosis, including *Rhipicephalus* (*R.*) *microplus*, *R. decoloratus*, *R. simus*, *R. evertsi evertsi*, *Dermacentor* (*D.*) *andersoni, D. variabilis*, *D. reticulatus*, *D. albipictus* and *Hyalomma* (*H.*) *marginatum rufipes* (Stiller et al. 1989; Samish et al. 1993; Jongejan and Uilenberg 2004; Kocan et al. 2004; Scoles et al. 2005, 2008; Zivkovic et al., 2007; Hove et al. 2018; Khan et al. 2022).

Other factors that may contribute to prolong the presence of *Anaplasma sp.* circulation include the possibility of infection to wild ruminants (Kocan et al. 2010), mobilization and introduction of infected cattle to a free herd, and some malpractice while doing procedures with contaminated instruments (Reinbold et al. 2010a; Zabel and Agusto 2018; Abdisa 2019; Bautista-Garfias et al. 2021).

The clinical effects of disease are more frequent in adult animals mobilized from a non-endemic herd to an endemic herd. Calves are often resistant to the pathogen presenting clinical sings of disease in rare occasions while adults are the group that present higher mortality rates at first contact with the pathogen (Rodríguez et al. 2009).

Life Cycle

Once *A. marginale* infect the erythrocytes, it replicates within them from one initial body to eight forming a type of morulae called inclusion body. The initial bodies contained in the inclusion body are released and multiply sequentially in other erythrocytes until high percentages of infection are reached. The initial bodies do not cause lysis of the erythrocytes when exit the bovine cells (Rodríguez-Camarillo et al. 2008). Prepatent period ranges from 7 to 60 days producing as many as 70% parasitezed red blood cells during acute presentation (Kocan et al. 2010). Instead of being destroyed by lysis in the blood vessels, macrophages in the spleen detect infected erythrocytes, and produces a diminution on the circulating cells (Kocan et al. 2004; Rodríguez et al. 2009). After acute phase has passed, bovines that recovers become carriers and may be a source of infection for other animals (Kocan et al. 2010).

When ticks feed from infected cattle they acquire the initial bodies present in erythrocytes (Fig. 1) and, once in the midgut of the tick, the initial bodies are released and infect the cells of the intestinal epithelium where they begin a cycle of development (Kocan et al. 2004). One (or more) cycles of *A. marginale* development in tick intestinal cells can pass and other tick tissues become infected (Cobaxin-Cárdenas et al. 2019), including salivary glands from where the pathogen is transmitted to vertebrates during feeding (Kocan et al. 2004). The first form of *A. marginale* seen within the colony is the reticulate (vegetative) form that divides by binary fission forming large colonies that may contain hundreds of organisms. The reticulate forms change to dense forms, which is the infective form and can survive outside the cells. Cattle become infected with *A. marginale* when the dense form is transmitted during tick feeding through salivary glands (Kocan et al. 2004).

Transmission

Anaplasma transmission in cattle occurs both biologically and mechanically. Biological transmission requires feeding of ticks on carrier animals while in mechanical transmission there is no multiplication of the bacteria in the vector and only infected blood is transferred from one animal to another (Foil, 1989; Kocan et al. 1981, 2010).

Biological Transmission

Several tick species have been associated with *Anaplasma* transmission, however, *D. andersoni* and *Rhipicephalus spp.* are the most studied (Kocan et al. 1992; Futse et al. 2003; Scoles et al. 2008). Transmission of *A. marginale* by *Dermacentor spp.*, *R. microplus* and *R. annulatus* have already been reported (Kocan et al. 2004). Biological transmission can be transstadial, from one stage to another, or intrasstadial, within the same stage (Stich et al. 1989; Samish et al. 1993; Ribeiro and Lima, 1996; Cobaxin-Cárdenas et al. 2019; Vimonish et al. 2020).

The relationship of *A. marginale* with ticks is complex and presents variations among different strains, vectors and environmental conditions. For example, it has been shown experimentally that only 10 infected ticks (*Dermacentor*) are enough to transmit *A. marginale* (Scoles et al. 2005). However, transmission has been experimentally described through *R. simus* ticks but was not achieved with *H. excavatum, R. sanguineus or R. annulatus.* Additionally, not all strains of *A. marginale* are transmitted efficiently by ticks (de la Fuente et al. 2001).

Intrasstadial and transstadial transmission are mainly important in three-host ticks, because they leave their host at the end of each stage. Until a while ago, it was considered that transovarial transmission of *A. marginale* did not occur in ticks; otherwise it has been reported that *R. microplus* larvae hatched from infected females were able to transmit the bacteria to cattle despite not having been fed previously. Transmission was confirmed molecularly by monitoring the variable region of *msp1a* (Amaro-Estrada et al. 2020) (Fig. 2). There are still several aspects to investigate, for example, temperature and strains involved seem to be relevant issues; but it is important to consider transovarial transmission in control strategies of bovine anaplasmosis.

Another form of transmission in cattle is transplacental transmission, which may occur during gestation, when the carrier cows suffer immunosuppression and an increase in rickettsemia occurs, allowing the bacteria to cross the placental barrier (Gale et al. 1996; Zabel and Agusto 2018). It has also been observed that some calves show clinical signs and do not survive, but others do not show signs but remain as reservoirs (Rey-Valeirón et al. 2003).

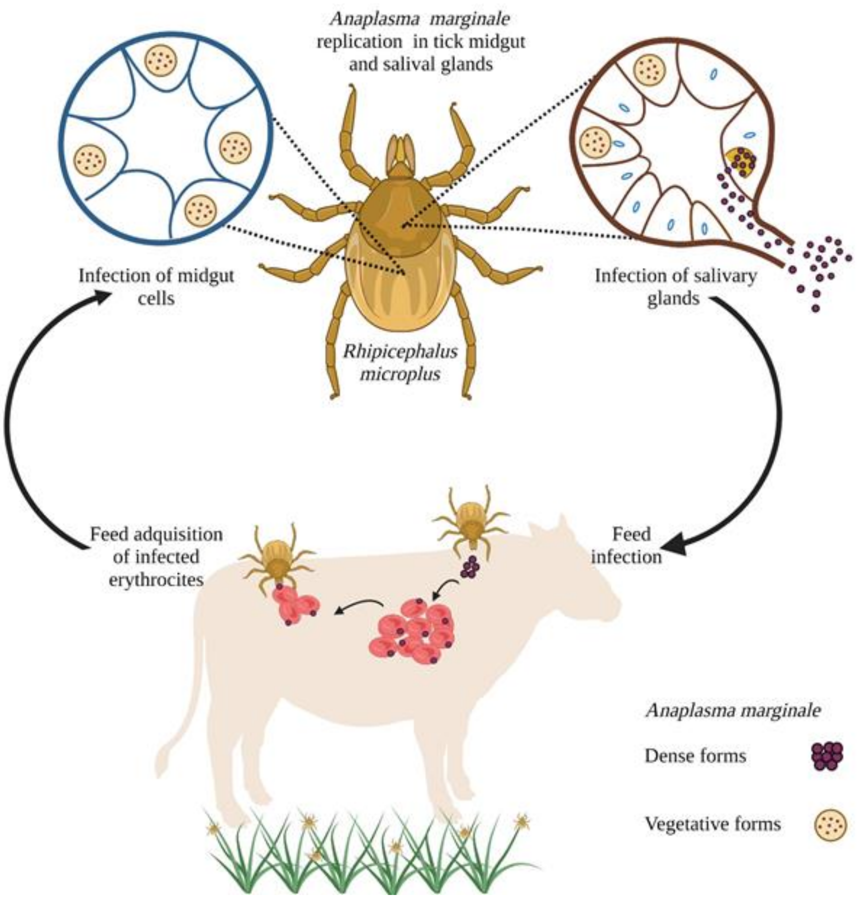

Fig. 1: Life cycle of *A. marginale* (made in Biorender.com under de license ZF250QYQMI).

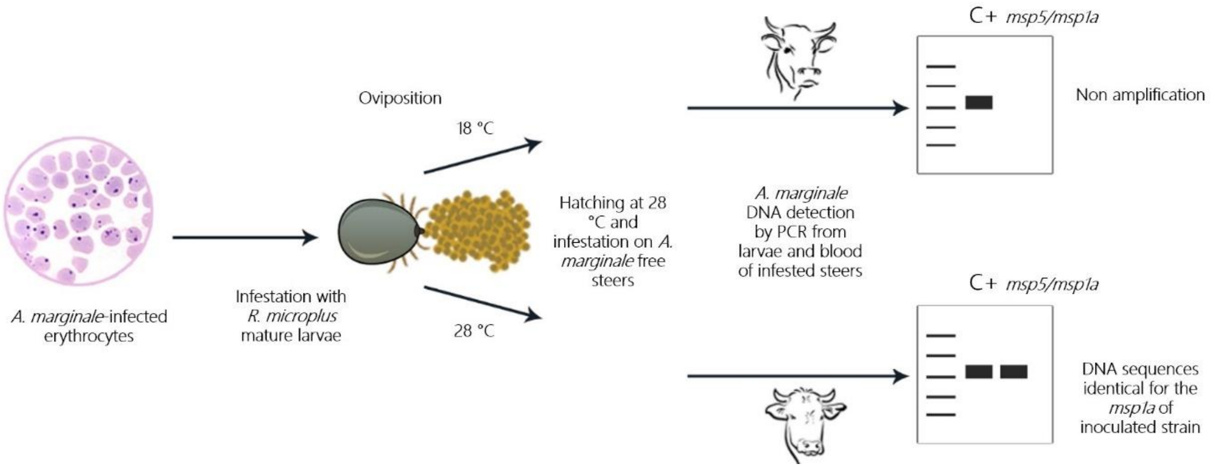

Fig. 2: Representation of transovarian transmission of *A. marginale* under controlled conditions.

Mechanical Transmission

Mechanical transmission of anaplasmosis occurs through contaminated fomites or when blood is transferred from one host to another. While blood sucking flies have shown a role of apparent little importance in the transmission of *A. marginale*, they can also participate in the horizontal transmission of bovine anaplasmosis in the absence of ticks (Kocan et al. 2010). Blood sucking flies are important as mechanical vectors of *A. marginale*, and by themselves as they feed on cattle's blood and lower the conversion efficiency due to disturbance in behavior of animal (Scoles et al. 2005).

Hematophagous arthropods such as *Tabanus bovinus and T. fuscicostatus* (horseflies), *Haematobia irritans* (horn fly) and *Stomoxys calcitrans* (stable fly) have been associated with infection, but appear to be at least 100 times less efficient than ticks fed on infected animals (Scoles et al. 2005; 2008). However, that low efficiency can be compensated by the large number of flies that can infest cattle (Bautista-Garfias et al. 2021).

Within the mechanical transmission, human malpractices does happen and probably accounts for many of the cases observed in the absence of ticks or where flies are under control. Vaccination with a single syringe and same needle, insertion of hormone implants and ear-tagging are customary practices in places where there are many animals (Kocan et al. 2010). It has been proven that needles used in procedures such as vaccinations are capable of transmitting *Anaplasma* from one infected animal to two subsequent ones (Reinbold et al. 2010a).

Bovine Immune Response

Infected erythrocytes are recognized by macrophages in reticular tissues and vascular sinusoids present in the red pulp of the spleen. The optimal capture activity by macrophages occurs until high concentrations of infected erythrocytes are reached (Rodríguez et al. 2008; Salinas-Estrella et al. 2022b). Non-specific phagocytosis has been demonstrated in the spleen and may explain splenomegaly present in the infections (Palmer et al. 1999).

When the erythrocyte is phagocytized, it initiates antigen processing by denaturing and partially digesting pathogen proteins into short peptides that are presented on MHC class II molecules on the macrophage surface to CD4+ T-helper (Th) cells (Brown et al. 1998a). Th cells involved in the immune response include Th1 cells, specialized in the production of IL2 and interferon gamma, which stimulate the synthesis of IgM and IgG2 synthesis by B cells and activate macrophages (Zaraza and Kuttler, 1971; Ristic and Carson, 1977; Arulkanthan et al. 1999; Rodríguez-Camarillo et al. 2008; Silvestre et al. 2018). However, there is also a response of Th2 cells that secrete IL4, IL6 and IL10. The cytokines also stimulate B cells towards the production of IgG1 (Rodríguez et al. 2008; Kocan et al. 2010). B cells can also be activated by recognizing an immunogen in its free, untransformed form through their antigen receptors or surface antibodies (Brown et al. 1998b; Zhao et al. 2016).

Studies on the different capacities of bovine immunoglobulin G isotypes indicate that IgG2 is related to the resolution of infectious processes (McGuire et al. 1991). This capacity has been confirmed in the case of bovine anaplasmosis, since these antibodies from immune animals are able to opsonize infected erythrocytes and be more rapidly phagocytized (Cantor et al. 1993), unlike IgG1 which is not able to mediate phagocytosis by neutrophils or monocytes (McGuire et al. 1991; Brown et al. 1998a). Cattle that recover from acute infection remain infected with low rickettsemia, but do not show severe clinical signs of the disease, so they are considered protected against a homologous challenge (Palmer et al, 1999; Rodríguez-Camarillo et al, 2008), except when there is a concomitant disease inducing a state of immunosuppression. The persistence of the parasite occurs despite the development of a protective immune response (French et al. 1998).

Diagnosis

Clinical diagnosis is based on the anamnesis, clinical signs, presence of vectors, cattle management practices, time of the year, and other aspects considered in the clinical history. Early diagnosis may improve the possibility of a good outcome and reduce the economic losses in an affected herd (Railey and Marsh, 2021). The disease is characterized by fever, lethargy, isolation, anemia, and a diminution of food intake, which, altogether, translates into a drop in the production (either meat or milk), affecting reproductive activity due to the effects of a low energy and oxygen transport (Rodríguez-Camarillo et al. 2008; Quiroz-Castañeda et al. 2016; Railey and Marsh, 2021).

When a presumptive diagnosis is made then it is necessary to confirm it with laboratory tests. The first option is microscopic observation of the pathogen in blood smears stained with Giemsa. Disadvantages of this technique include; 1) the person analyzing the smear should have expertise in it 2) moment of the cycle during which sample is analyzed as a sample taken at the beginning or the end of the infection may not be quantifiable (Barigye et al. 2004; Kocan et al. 2010; Railey and Marsh, 2021).

Serological tests are recommended in different scenarios: a) for monitoring the development of antibodies at first infection or immunization; b) for epidemiological purposes: either when the herd is stable and located at an enzootic region, or when an outbreak occurred; c) in occasions when trading exchange requires it in order to mobilize only free of contact bovines (Rodríguez Camarillo et al. 2008; Rodríguez et al. 2009; Salinas-Estrella et al. 2022a).

The molecular diagnosis facilitates the identification of pathogen, when the infection is not quantifiable at microscope. It also allows the procurement of DNA for genomic identification. Several genes are used as target for molecular amplification by PCR including *msp5* gene as the elected gene for standard detection, followed by *msp1a, msp4* (Torioni de Echaide et al. 1998; Rodríguez et al. 2009; Singh et al. 2012; Bacanelli et al. 2014; Amaro-Estrada et al. 2020). Other molecular test have been developed for *A. marginale* detection, such as multiplex PCR (Figueroa et al. 1993), qPCR (Carelli et al. 2007), duplex qPCR (Decaro et al. 2008), PCR followed by restriction fragment length polymorphisms (PCR-RFLP) (Noaman et al. 2009), reverse line-blot hybridization (RLBH) (Guillemi et al. 2015) and loop-mediated isothermal amplification (LAMP) (Giglioti et al. 2019).

Control Strategies

Control of bovine anaplasmosis includ the actions towards control of tick-vector and immunoprophylaxis. The eradication of ticks has not been very practical as intense dipping or spraying with acaricides has resulted in resistance and failure in effectivity (Rodriguez-Vivas et al. 2017). According to some studies, rotational grazing is a controversial practice as it works best if pastures are left fallow for at least 60 days (Nicaretta et al. 2020), while some other studies proved that its success depends on extensive knowledge of local climatic conditions, plus robust data on seasonal peaks in tick populations (Hüe et al. 2021). Therefore, to be successful, it is important to apply grazing management whenever there is sufficient robust data from the area (Hüe et al. 2021).

Horizontal transmission due to blood transfer from one animal to another by flies can be reduced by a number of management practices, such as the use of repellents, insecticides, biopesticides, manure management, traps, etc. (Scoles et al. 2005; Cook 2020).

Iatrogenic transmission is one of the most unattended forms of dispersion of diseases. Changing needles between animals, disinfection of ear-taggers and other tools that get in contact with blood are the most simple and economical practices to curtail this form of horizontal transmission (Reinbold et al. 2010a).

On the immunoprophylaxis spectrum, there have been many attempts to induce protection against disease. "Premunition" is described as the inoculation of whole or diluted blood into adult or young animals (Todorovic et al. 1975). Inoculation of blood from animals with ongoing clinical disease is a dangerous option and close monitoring is critically important as it usually induces a severe clinical syndrome which may be fatal. Diluted blood from an infected carrier or a patent animal can be used, but the number of infected erythrocytes is unknown and the monitoring period varies from one inoculation to the next (Todorovic et al. 1975). Best results from practice are observed when calves between the ages of 6 and 9 months are the recipients of the infected blood (Kuttler 1979). Animals immunized through this way usually develop a sub-clinical syndrome that protects the recipient as long as the animal remains infected. The procedure should preferably be carried with local strains. *A. centrale* has been used in many countries but in México, it is banned as it is considered as an exotic pathogen (Rodríguez et al. 2009). Close monitoring in clinical variables but specifically packed cell volume (PCV) and vaginal mucosa coloration (VMC) in dairy calves, correlate with presence of bovine anaplasmosis, thus these parameters can be used as guide to apply timely treatment and significantly reduce losses in valuable dairy stock (Heller et al. 2022).

Treatment of bovine anaplasmosis can be done with two basic antibiotics, oxytetracycline and imidocarb dipropionate (Coetzee et al. 2005; Reinbold et al. 2010b; Sarli et al. 2021).

Vaccine Developments

The first description of *A. centrale* as a live immunogen (Theiler 1911) demonstrated that it can ameliorate the clinical signs of *A. marginale* infection. This strain has been used in many countries for the prevention of bovine anaplasmosis (usually as trivalent including *Babesia* (*B.*) *bovis, B. bigemina and A. centrale*). There are 23 complete genome sequences of *A. marginale* available at NCBI which can be used to design a vaccine that can be applied in animals of all ages and protect against many strains that have been reported yet (Rodríguez-Camarillo et al. 2020). Attenuation of naturally pathogenic strains was one of the first efforts to generate immunity, but some of these strains were passed into "unnatural" hosts such as deer or sheep with variable results (Zaraza and Kuttler, 1971; Kuttler and Zaugg, 1988). Strains of *A. marginale* of naturally low virulence have also been tested (Bock et al. 2003; Rodríguez-Camarillo et al. 2008) with promising results, yet not a single one of these options has reached the commercial use, because of the ever-present risk of transmission of blood associated pathogens with implicit disastrous results (Rogers et al. 1988). Inactivated vaccines tested so far while inducing solid immunity to homologous challenge, are yet to induce the same level of protection against heterologous strains (Salinas-Estrella et al. 2022b). Proteins that in their native form have induce solid immunity have failed to induce immunity as recombinant counterparts (Sarli et al. 2020) and even in some cases, animals inoculated with them showed worse clinical syndromes (Ducken et al. 2015). An ear implant was designed to deliver *Msp1a* tandem repeat K;S as the antigen in a scheme that comprised three vaccination doses (prime-boost like) (Curtis et al. 2020).

As mentioned before, live organisms would seem to induce complete immunity against homologous and heterologous challenge. Based on this premise, targeted mutagenesis was used for deletion of the phage head-to-tail connector protein (phtcp) gene in *A. marginale* (that is cultivable on tick cells) (Hove et al. 2022). The resulting mutant did not cause disease and exhibited attenuated growth in its natural host (cattle). When its ability to confer protection against wild-type *A. marginale* was challenged, all animals receiving the live mutant did not develop clinical signs or anemia, or erythrocyte infection. In contrast, animals immunized with whole cell inactivated vaccine developed similar disease as the non-vaccines following *A. marginale* infection (Hove et al. 2022). This is very innovative approach as the organism is cultivable on tick cell lines, thus it is not exposed to cattle pathogens and cannot transmit them and it can be reproduced on demand. The mutation seems to be stable, thus it will not reverse the virulence in the host and finally it may protect a wide array of heterologous strains upon testing (Hove et al. 2022).

Perspectives

New diagnostic techniques will need to be developed or implemented at ranch-site if we want to treat an animal on the spot, when no microscope or serology result is available and the animals is suffering with the clinical symptoms of anaplasmosis (Giglioti et al. 2019; Salinas-Estrella et al. 2022a,b).

In reference to the treatment, two different oxytetracycline-resistance genes (otrA, and otrB) were detected recently by PCR in samples from infected cattle and sheep with *A. marginale* in the Northwest and Southwest of Iran (Shahbazi et al. 2021). While this discovery opens the possibility of oxytetracycline resistance, there have been no reports of such resistance when treating bovine anaplasmosis. What is known is that long-term treatment of *Anaplasma* carriers with oxytetracycline does not necessarily result in elimination of the carrier state (Curtis et al. 2020). This is an area of clinical interest as resistance to oxytetracyclines could be the next great challenge when treating cattle against anaplasmosis in the absence of an effective vaccine (Shahbazi et al. 2021).

Regarding immunoprophylaxis, there are numerous studies aimed at inducing long-term immunity against bovine anaplasmosis. Molecular diversity and antigenic variability have proven to be an obstacle to the induction of protective immunity against all strains studied so far. Although studies have been conducted with immunorelevant proteins, these proteins may not be conserved in all strains, so that robust protection may be induced against some strains but not others (Quiroz-Castañeda et al. 2016; Salinas-Estrella et al. 2022b). Native, recombinant proteins and peptides have been tested as immunogens, with results that range from full protection against homologous challenge to no protection or even a worse clinical syndrome against heterologous strains (Ducken et al. 2015; Curtis et al. 2020; Sarli et al. 2020).

Live *A. centrale* vaccines are still used in many countries, sometimes with disastrous results in terms of transmission of other blood-borne pathogens (Rogers et al. 1988). Still, live agents that can establish a subclinical infection, may seem to be one of the best alternatives for induction of solid protection. Therefore, genetically modified *A. marginale* tested in susceptible cattle may be one of the best options, as it can be grown on tick cell lines and is not in contact with bovine pathogens, therefore unable to transmit them. It can be produced on demand, and is likely to protect against heterologous strains (Hove et al. 2022).

An effective vaccine is a demand from cattle producers in many countries that perhaps can now be fulfilled with mastered techniques; even if is not a universal vaccine it can be applied to local strains that can more fully cover the antigenic spectrum of a region and be produced by government laboratories for the benefit of each country's cattle industry (Salinas-Estrella et al. 2022b).

Conclusion

Bovine anaplasmosis is a widely distributed and economically important infectious disease. To know the clinical signs, the economic effects, the diagnostic and treatment options and the several attempts to develop a protective universal vaccine gives a broad description of the problem this disease represents for bovine health and production around the world. Its control has been tried multiple times since its discovery in 1910. Several approaches are still in progress to develop effective control strategies. With the climate change and its effects on the habitats in the last years, studies on the distribution of biological vectors and the participation of new species pf ticks as vectors has to be made in order to determine the best course of action to control and prevent bovine anaplasmosis.

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15

New Bacterial Species Resistant To Antibiotics, Current Situation

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INTRODUCTION

According to various international institutions, antimicrobial resistance (AMR) is such a severe problem that, within 30 years, it could cause the death of more people than those affected by chronic problems such as cancer. The World Health Organization indicates that (World Health Organization, 2015), that AMR is responsible for up to 700 000 deaths worldwide, which could increase up to 10 million deaths annually. In the United States of America, up to 2 million people contract infections associated with AMR bacteria annually leading to the death of almost 23,000 people (Dadgostar 2019). AMR affects not only mortality but also morbidity. It triggers a high economic burden and more extended periods of hospitalization and on a large scale, it causes economic losses in healthcare systems worldwide (Shrestha et al. 2018; Christaki et al. 2020). Statistics show that in 30 years, there may be a reduction of up to three percent of the Gross Domestic Product due to antimicrobial resistance, impacting the world with a loss of up to $100 billion. (Shrestha et al. 2018; Dadgostar 2019). However, these data might be underestimated, as they only consider a subset of drug-resistant bacteria due to the lack of available data on emerging resistant bacteria (Rodríguez-Medina et al. 2019).

When bacteria escape the drug's effect due to the development of cellular mechanisms of response to the aggression, it is reffered as antibiotic resistance (Jubeh et al. 2020). One of the operational definitions of antimicrobial resistance indicates that a strain has antimicrobial resistance if its minimum inhibitory concentration is higher than that exhibited by its similar wild-type strain (Martínez et al. 2015).

Each antimicrobial agent has a unique mode of action that depends on two fundamental aspects which include bacterial cell characteristics and antibiotic targets. Regarding bacterial cell characteristics, differences are distinguished between Gram stain-positive and Gram stain-negative bacteria. Although the structures of both are similar, there are some critical discrepancies. Gram-negative bacteria possess an outer membrane that confers resistance to a high number of antibiotics as it is one of the main targets of their mode of action (Assoni et al. 2020). Alterations in their hydrophobicity properties and changes in porins or lipopolysaccharides contribute to the potential for resistance (Jubeh et al. 2020).

Gram-positive bacteria lack an outer membrane but the presence of thick peptidoglycan layers dominates their anatomy. Lacking the outer membrane, they are more sensitive to the effect of antibiotics. That is why Gram stain-negative species show a higher frequency of resistance and are resistant to more antibiotics (Jubeh et al. 2020). Some agents act against both types of bacteria. These are known as broad-spectrum antimicrobials (Bearden and Danzinger 2001).

Antimicrobial agents can interfere with cell wall synthesis, protein synthesis, nucleic acid synthesis or inhibit a metabolic pathway. Bacteria, for their part, counteract these effects through mechanisms such as 1- intrinsic resistance, a natural property of each bacterial group; 2- acquired resistance, a trait that is a direct function of bacterial genetic variability and may be due to mutations and horizontal gene transfer; 3- Genetic changes in DNA, also called mutational resistance, involve modification of the drug's mode of action, e.g., decreased absorption, activation of exit mechanisms to extradite the harmful molecule or global changes in critical metabolic pathways; and 4- horizontal gene transfer (transformation or conjugative transduction) (Munita and Arias 2016).

Microorganisms possess intrinsic resistance to one or more antimicrobials naturally. The problem occurs when they generate acquired resistance in clinical settings, which causes a bacterial population that was initially susceptible to an antimicrobial to subsequently no longer be so, causing morbidity and mortality (Paterson 2006; Ruppé et al. 2015 2015; Jubeh et al. 2020).

Causes of Antibiotic Resistance

Intrinsic resistance has been well known since the discovery of penicillin. Before its use, the first resistant strains of *Staphylococcus* had already been described. Subsequently, methicillin was introduced, and soon after, a resistant strain

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was reported (Sengupta et al. 2013) and to counter its ineffectiveness, vancomycin was introduced and after two decades later, *Staphylococcus* resistant was reported (Barberato-Filho et al. 2020).

Enabling elements for acquired resistance include misuse and overuse of antibiotics (Zaman et al. 2017; Chokshi et al. 2019; Dadgostar 2019), agricultural use (Chang et al. 2015), rising income levels conducive to overconsumption (Chaw et al. 2018; Klein et al. 2018), travel routes exposing humans to resistant pathogens and their dissemination in various countries, as well as lack of knowledge creating a gap in awareness of antibiotic use globally (Frost et al. 2019).

For this reason, international bodies have launched guidelines that aim to help and safeguard the efficacy of antimicrobials (WHO, 2015, 2022; McEwen and Collignon 2018). The World Health Organization (WHO) classified AMR bacteria that posing an imminent threat to human health (De Oliveira et al. 2020) and published a list of priority pathogens that require urgent research and development of new treatments (WHO 2017).

Priority Bacteria with Antibiotic Resistance

The critical priority group includes multidrug-resistant bacteria that are of great attention because they affect hospitalized patients requiring devices such as catheters and ventilators. The high and medium priority categories contain bacteria with increasing drug resistance (WHO 2017) as mentioned in Table 1.

In addition to the priority resistance determined by the WHO, the bacteria included in the list present resistance profiles to other drugs, which shows their capacity to develop diverse resistance mechanisms. Within this list, we can locate a group of pathogens with a high capacity to escape the antimicrobial effect under the acronym "ESKAPEE": *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp. and *Escherichia coli* (Arato et al. 2021; Mancuso et al. 2021) as mentioned in Table 2.

New Bacterial Species Resistant to Antibiotics

The evolution of antimicrobial resistance and its spread and appearance in diverse ecosystems is due, among other factors, to the interconnection between animal, human and environmental habitats. The spread of resistant clones and antibiotic resistance determinants has been described among microorganisms that previously did not exhibit this characteristic. From this derives the concept of "emerging antibiotic-resistant pathogens," defined as those microorganisms that have recently developed antibiotic resistance, affecting a population by rapidly increasing their incidence or geographic range (Vouga and Greub 2016). Among these, we can find the following:

Klebsiella variicola

It is found within the *Klebsiella pneumoniae* complex and in 2004, it was described as a new species (Wyres et al. 2020). Its natural niches are plants; however, the most current reports show an increasing incidence of strains of clinical origin with AMR (Rivera-Galindo et al. 2021), implicating it in bacteremias, infections of the respiratory system, and urinary tract infections in humans. Therefore, it is considered as an emerging pathogen (Srinivasan and Rajamohan 2020).

One of the main variables to consider when studying antibiotic-resistant strains is the correct identification of the strain to be able to carry out an adequate epidemiological follow-up. In the case of *Klebsiella* (*K.*) *variicola*, the biochemical tests routinely used in the clinical microbiology laboratory or automated systems worldwide has resulted in its misclassification as *K. pneumoniae*. (Long et al. 2017; Fontana et al. 2019; Piepenbrock et al. 2020; Kiley et al. 2021; Rivera-Galindo et al. 2021). Derived from this, there is scarce data on its susceptibility patterns, epidemiological characteristics of distribution in the population, and its actual clinical implications (Rodríguez-Medina et al. 2019).

The few existing reports indicate that it is intrinsically resistant to ampicillin due to the chromosomal LEN β-lactamase (Rodríguez-Medina et al. 2019; Morales-León et al. 2021). It is a carbapenemase-producing species. It is resistant to ertapenem, meropenem and imipenem (Hopkins et al. 2017). Recently, its resistance to colistin, mediated by chromosomal mechanisms, was reported (Jayol et al. 2017; Lu et al. 2018). Likewise, there is evidence of horizontal gene transfer between members of the complex as they have been found to share plasmids, which favors the spread of AMR genes. Because of this, accurate identification is essential (de Campos et al. 2021).

Mycobacterium abscessus

It is a fast-growing, multidrug-resistant, nontuberculous mycobacterium species that has recently become a significant threat to people with chronic lung conditions (Bryant et al. 2021). Infection rates caused by this species are increasing globally, likely due to its dispersal via aerosols and spread through fomites (Bryant et al. 2016). Its intrinsic resistance mechanisms are due to a highly impermeable cell envelope, multidrug exit pumps, and the ability to encode several enzymes that can inactivate antibiotics (Nessar et al. 2012; Luthra et al. 2018; Gorzynski et al. 2021).

Due to extensive, repeated, or inappropriate use of antimicrobials, most strains of this species are resistant to macrolides due to the expression of an erythromycin ribosome methylase gene (erm) (Nessar et al. 2012; Luthra et al. 2018; Lopeman et al. 2019). They are also resistant to aminoglycosides, due to the presence of a mutation in the rrs gene responsible for coding for the 16S rRNA (Johansen et al. 2020). They are resistant to beta-lactams due to the presence of class A beta-lactamase.

Table 1: Priority bacteria with antibiotic resistance, according to WHO (2017)

Priority level	Name	Priority resistance
Critic	*Acinetobacter baumannii,*	Carbapenem-resistant
	Pseudomonas aeruginosa,	Carbapenem-resistant
	Escherichia coli	Carbapenem-resistant, extended-spectrum beta-lactamase (ESBL)-producing carbapenemics
	Klebsiella pneumoniae	
	Enterobacter sp	
High	*Enterococcus faecium*	Vancomycin-resistant
	Staphylococcus aureus	Methicillin-resistant, with intermediate sensitivity and vancomycin resistance
	Helicobacter pylori	Resistant to clarithromycin
	Campylobacter spp	Resistant to fluoroquinolones
	Salmonellae	Resistant to fluoroquinolones
	Neisseria gonorrhoeae	Cephalosporin-resistant, fluoroquinolone-resistant
Medium	*Streptococcus pneumoniae*	Penicillin resistant
	Haemophilus influenzae	Ampicillin resistant
	Shigella spp	Resistant to fluoroquinolones

Table 2: Resistance characteristics of bacteria named ESKAPEE (De Oliveira et al. 2020)

Priority level	Name	Priority resistance	Resistance to other antimicrobials	Mechanism of resistance	Reference
High	*Enterococcus faecium*	Vancomycin	Ampicillin, penicillin, cephalosporins, vancomycin and aminoglycosides such as tobramycin, kanamycin, gentamicin, and fluoroquinolones.	Chromosomal gene pbp5 encodes a class B penicillin-binding protein, aminoglycoside-modifying enzymes, enzyme modification, and ribosomal target modification.	(Emaneini et al. 2008; Cattoir and Giard 2014; Novais et al. 2016; Gorrie et al. 2019).
High	*Staphylococcus aureus*	Methicillin, with intermediate sensitivity and resistance to vancomycin.	Fluoroquinolones	Plasmid-encoded penicillinase, penicillin-binding protein, a mutation in genes encoding target enzymes for DNA replication	(Chambers and Deleo 2009; Tanaka et al. 2000)
Critical	*Klebsiella pneumoniae*	Carbapenemics and extended-spectrum beta-lactamase producer.	Multiresistant	Plasmid accessory genomes and chromosomal gene loci	(Cifuentes-Castaneda et al. 2018; Nakamura-Silva et al. 2022)
Critical	*Acinetobacter baumannii,*	Carbapenemics	Tigecycline, aminoglycosides, colistin	Production of four β-lactamases (A, B, C, D) exit pumps, three classes of enzymes, including acetyltransferases, adenylyltransferases, and phosphotransferases, and loss of lipopolysaccharide.	(Lee et al. 2017; Trebosc et al. 2019; De Oliveira et al. 2020)
Critic	*Pseudomonas aeruginosa*	Carbapenemics	Multidrug resistant	Overexpression of exit pumps and decreased outer membrane permeability, genes encoding for porins and other protein β-lactamases class A, C, and D, aminoglycoside-modifying enzymes.	(Langendonk et al. 2021; Mancuso et al. 2021)
Critic	*Enterobacter sp*	Carbapenemics and extended-spectrum beta-lactamase producer	Fluoroquinolones and aminoglycosides	β-lactamases type A and type B.	(Davin-Regli et al. 2019)
Critic	*Escherichia coli*	Carbapenemics and extended-spectrum Beta-lactamase producer	Broad-spectrum cephalosporins, polymyxins, Fluoroquinolones	Carbapenemases aminoglycoside, 16S rRNA methylases, mcr genes.	(Raphael et al. 2021; Wu et al. 2021; Rodríguez-Avial et al. 2013; Jayol et al. 2017; Sadecki et al. 2021).

They also show resistance to tetracyclines due to enzymatic inactivation by flavine-adenine dinucleotide inactivator monooxygenase (Nessar et al. 2012; Ananta et al. 2018; Luthra et al. 2018; Victoria et al. 2021). As for fluoroquinolones, their resistance to these compounds is due to a mutation in the quinolone resistance determinant region (Johansen et al. 2020).

Staphylococcus xylosus

It is a coagulase-negative *Staphylococcus* that is implicated in animal infections. However, due to the reports of its ability to produce infections in humans; it is considered an emerging pathogen (Qu et al. 2021).

Human infections include brain abscesses, pyelonephritis, endocarditis, and septicemia. In addition, it is increasingly reported as a nosocomial infection-producing species. At the clinical level, it is possible to detect its resistance to macrolides, and it has been determined that this is due to the presence of the erm gene (Yuan et al. 2021).

Multidrug-resistant strains can also be found, i.e., with resistance to several families of antibiotics. We can consider in this sense that it presents resistance to lincosamide, tetracyclines, and aminoglycoside. There is little information on this species and its novel multidrug resistance, so accurate identification and monitoring should be corroborated with current epidemiological data (Wipf et al. 2017).

Elizabethkingia anophelis

This species is associated with human diseases, especially neonatal nosocomial outbreaks and increasing incidence of bacteremia and mortality (Huang et al. 2017). Recently, it has been a leading cause of life-threatening infections in Hong Kong, the United States, and Taiwan (Spurbeck and Arvidson 2010; Lau et al. 2016; Perrin et al. 2017; Choi et al. 2019; Wang et al. 2019). It is considered as an emerging opportunistic pathogen and is often misdiagnosed because automated identification systems routinely used at the public health service level lack sufficient data for its detection (Lin et al. 2018).

It is intrinsically resistant to many antimicrobial agents commonly used to treat Gram-negative infections, such as carbapenem, cephalosporins, and colistin. It was recently reported to exhibit resistance to at least 20 antibiotics due to genes encoding different beta-lactamases and efflux pumps (Wang et al. 2019). Isolates are usually resistant to cephalosporins, carbapenemics, aminoglycosides, fluoroquinolones, and vancomycin (Teng et al. 2021). Mutations in quinolone resistance determinant regions and amino acid alterations have been detected to be associated with levofloxacin resistance (Jian et al. 2018; Lin et al. 2018).

Escherichia fergusonii

It was classified in 1985 as a new species (Farmer et al. 1985). It is an opportunistic pathogen initially associated with septicemia and diarrhea in animals but is now associated with abdominal wounds, urinary tract infections, and bacteremia in humans (Tang et al. 2022). Recent reports consider it a species of great importance because it frequently affects neonates in intensive care units (Rivera-Galindo et al. 2021). Phenotypic methods generally identify it as *E. coli*; becauseat the epidemiological level, there is an underreporting of pathogenic microorganisms and that effective methods for their detection and treatment are not developed (Tang et al. 2020; Rivera-Galindo et al. 2021).

Escherichia (*E.*) *fergusonii* emerges as a microorganism of concern due to its potential for multidrug resistance. It is a producer of broad-spectrum beta-lactamases resistant to carbapenems (Tomilola et al. 2019). In 2016, a plasmid-borne resistance gene was identified as the primary factor contributing to its colistin resistance (Zhi et al. 2016; Wang et al. 2018; Tang et al. 2020; Liu et al. 2022).

Importantly, there is very little information on the pathogenic potential of this species in humans. However, information on its resistance mechanisms to strains isolated from animals is high; so, we can consider it an emerging zoonotic pathogen (Tang et al. 2020; Guan et al. 2022; Liu et al. 2022; Shah et al. 2022; Tang et al. 2022). As addressed above, the interconnectedness between human, animal and environmental habitats is conducive to the emergence, evolution, and spread of resistance, so the evolution of this species and those described above should be closely monitored.

Conclusion

Since many studies and clinical practice continue to rely on traditional methods based on bacterial culture and automated systems to identify nosocomial antibiotic-resistant microorganisms, it is imperative to show the incidence of new resistant bacterial species emerging as potential health problems. With this information, reference databases can be updated, and the need to migrate towards molecular techniques for accurately identifying emerging microorganisms which can be evidenced whenever possible. The increasing presence of antibiotic-resistant pathogenic species shows the need to minimize the use of inappropriate antimicrobial therapies as they represent risk factors for morbidity, mortality, and economic impact related to health care. If bacteria are accurately identified, epidemiological and clinical studies can make significant advances so that, in the short term, clinicians can prescribe targeted antibiotics that promote the reduction of antimicrobial resistance.

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16

History, Current Situation, and Future Perspectives on Antibiotics and Antibiotic Resistance

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INTRODUCTION

Antibiotics are the most crucial drugs for fighting bacterial infection, widely used to treat and prevent such diseases. Even though these are used against bacterial infections, some antibiotics also possess antiprotozoal activity. The term *antibiotic* means "opposing life," from the Greek roots *anti*, "against," and *bios*, "life," and refers to any compound used against germs (Hutchings et al. 2019).

The records of using antibiotics date back to ancient times. Many civilizations from ancient Egypt, Nubia, China, Serbia, Greece, and Rome topically applied moldy bread to treat infections. John Parkinson (1567–1650) was the first to document infection treatments with molds (Hutchings et al. 2019). Moreover, antibiotics overturned medicine in the 20th century when Alexander Fleming (1881–1955) came upon penicillin in the late 1920s, and the widespread use of which significantly reduced the wartime death rates (Hutchings et al. 2019).

Antibiotic classification can be based upon the mechanism of action as the inhibitors of bacterial cell wall synthesis, protein synthesis, nucleic acid synthesis, folate metabolism, and cell membrane disruptors. Another way to classify antibiotics is according to the spectrum of activity into narrow- and broad-spectrum antibiotics. A narrow-spectrum antibiotic can only kill or inhibit limited bacterial species. On the other hand, a broad-spectrum antibiotic affects two major bacterial groups, Gram+ve and Gram-ve (Joyner et al. 2020).

The continuous use of antibiotics eventually leads to resistance development by infectious bacteria (Christaki et al. 2020). Antibiotic resistance (AR) is the loss of bacterial susceptibility to an antibiotic's bactericidal or bacteriostatic properties (Frieri et al. 2017). AR refers to the increased tolerance to therapeutic regimens to which the pathogen was sensitive before developing resistance. When a resistant strain dominates an infection, it may be untreatable and grievous. Bacterial pathogens have been a significant cause of illness and fatality for a long time. The antibiotic discovery provided a simple and efficient therapy for bacterial infections, and since then, antimicrobials have tremendously impacted human wellness and longevity. However, these are jeopardized by the rise of AR as many infective bacteria have become resistant to the main antimicrobial classes, and multidrug-resistant microbes have caused untreatable diseases (MacLean and San Millan 2019).

AR poses significant health and economic effects, and the yearly cost could rise to 10 million deaths and US$100,000 billion by 2050 (MacLean and San Millan 2019). AR development was reported in the early 1940s following penicillin's initial large-scale uses. Penicillin's massive production was part of World War II's bigger war campaign when the drug was utilized widely by military units and some civilian people (Banks et al. 2019). With penicillin's efficacy in treating wounds, the antibiotic was exalted for reducing sexually transmitted diseases among the troops since it was effective against bacterial pathogens famous for causing syphilis and gonorrhea (Guy 1991).

Penicillin resistance was reported even before the Second World War—firstly, in 1940 by British scientists Chain and Abraham, who reported an enzyme able to destroy the drug. In 1944, several researchers independently described a penicillin-deactivating enzyme released by some microbes (Lima et al. 2020). In the subsequent decades, overexploitation and repeated antibiotic use favored the survival and emergence of many antibiotic-resistant bacteria (Chokshi et al. 2019).

Bacterial cells can acquire AR in two main ways: through mutations in the cell's DNA during replication and resistance acquisition by bacteria through horizontal gene transfer (McInnes et al. 2020). These mechanisms give rise to resistance development since they cause biochemical changes that alter specific bacterial cell properties, rendering the cell susceptible to an antibiotic. These mechanisms include activating efflux pumps that take a drug out of the cell, drug-inactivating enzymes, altering bacterial molecular drug targets, and inhibiting drug uptake (Yelin and Kishony 2018).

Antibiotics are essential for treating medically significant disease cases and are used in animals to treat and prevent infections. Indiscriminative use of antibiotics or incomplete treatment courses causes resistant bacteria to tolerate a sublethal drug dose. This is the leading reason for drug-induced resistance that distributes among humans via food,

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water contaminated by animal waste, and the surroundings where slaughtered animal waste is discarded. The resistance can affect the human population, causing huge socioeconomic costs (Ahmad and Khan 2019).

History of Antibiotic Development

Molecules with antibiotic effects synthesized by various microbes were present long before humanity realized their benefit in treating bacterial diseases. Microbes have been exposed to selection pressures from antimicrobials for a long time, mainly within small areas where antibiotic-producing organisms exist. Mass-production of antibiotics started in the 1940s, mainly semisynthetic products of naturally synthesized molecules and a few completely synthetic antibacterials. Consequently, bacterial populations became exposed to high antibiotic concentrations, leading to speedy resistance among many pathogens today (Chinemerem Nwobodo et al. 2022).

The golden years of antibiotic discovery, development, and production took place from 1940 to the 1960s and continued in the following years but not as speedily as in previous years. The timeline of antibiotic development is depicted in Fig. 1. The most common antibiotics are penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, chloramphenicol, and glycopeptides. Alexander Fleming came upon the first antibiotic, penicillin G, in 1928. Penicillin is a bactericidal drug that acts by adhering the β-lactam ring to DD-transpeptidase. This binding prevents peptidoglycan cross-linking and new cell wall synthesis (Stepek et al. 2019).

Cephalosporins are also β-lactam bactericidal antibiotics discovered by the Italian scientist Giuseppe Brotzu in 1945 by isolating a mixture of compounds from *Acremonium*, a mold previously called *Cephalosporium* (Jones 2008).

In 1944, a *Streptomyces griseus* strain was found to synthesize an aminoglycoside named streptomycin, which prevents protein production by attaching to the bacterial 30S ribosomal subunit. Streptomycin marketing began in 1946 to treat tuberculosis, tuberculous meningitis, and other bacterial infections (Willey et al. 2008).

Chloramphenicol was initially purified from a culture of *Streptomyces venezuelae* in 1947. Chloramphenicol's antimicrobial effect is a reversible binding to the ribosomal 50S subunit, preventing bacterial protein synthesis. Chloramphenicol was the first Food and Drug Administration (FDA)-approved broad-spectrum antibiotic, showing excellent tissue permeability. Nevertheless, various toxicity issues were discovered in the 1960s, and it is presently seldom prescribed (Wiest et al. 2012).

The introduction of tetracyclines started with the discovery of chlortetracycline in 1945. Members of this class exert antibiotic activity by disrupting protein synthesis and acting on the ribosomal 30S subunit. Chlortetracycline, a product of *Streptomyces aureofaciens*, is unstable in strong basic or acidic solutions, leading to its hampered bioavailability (Liu and Myers 2016).

Macrolides are the second most commonly prescribed antibiotics after penicillins. These are naturally synthesized by *Saccharopolyspora erythraea* and were discovered in 1952, starting with erythromycin. Erythromycin is a bacteriostatic antibiotic that binds to a bacterial 50S ribosomal subunit. However, its poor oral bioavailability and instability under acidic conditions hinder its therapeutic use (Cyphert et al. 2017). Virginiamycin, isolated from *Streptomyces virginiae* in 1952, was the first identified streptogramin, a class of antimicrobials formed by a polyunsaturated macrolactone and a cyclic hexadepsipeptide. Either substance binds to the ribosomal 50S subunit, demonstrating a middling activity, but their synergistic effect increases their therapeutic efficacy (Mast and Wohlleben 2014). Fig. 1 indicates the timeline regarding the discovery of various antibiotics.

The first glycopeptide, vancomycin, was discovered in 1956 as a product of *Amycolatopsis orientalis* and is currently a last-line drug. Glycopeptides interrupt cell wall synthesis by interfering with the transpeptidation and transglycosylation steps, inhibiting cross-linking and cell wall maturation (James et al. 2012).

Classification of Antibiotics

The ribosome is one of the best antibiotic targets, and many antimicrobials used presently specifically target the bacterial ribosome. Many antibiotics suppress bacterial growth by inhibiting ribosome functions. For example, macrolides are broad-spectrum bacteriostatic antibiotics inhibiting many Gram+ve bacteria, used for over sixty years. Macrolides hinder bacterial protein synthesis by binding to the ribosomal 50S moiety and targeting the bacterial ribosomal nascent peptide exit tunnel (NPET). The NPET is about 100 A° long and 10–20 A° in diameter and is a hallway that allows synthesized proteins to leave the ribosome (Vázquez-Laslop and Mankin 2018).

Macrolides were thought to inhibit translation merely by obstructing the NPET and inhibiting the movement of newly synthesized polypeptides once they reached a certain length. Structural studies have supported this view by exhibiting that macrolides significantly narrow the NPET (Fig. 2). This concept was confirmed by *in vitro* studies demonstrating that erythromycin causes peptidyl-tRNA accumulation during synthetic mRNAs' translation, suggesting translation disruption in its early stages (Seefeldt et al. 2021). Peptidyl-tRNA accumulation was also detected in macrolide-treated cells (Hasan et al. 2021).

Aminoglycosides are bactericidal antibiotics that bind to the ribosomal 30S subunit. These are thought to adhere to the A-site (aminoacyl) on the 16S rRNA, which is a portion of the 30S ribosomal subunit. This binding causes the genetic code to be misread and disruption of translation, leaving the bacteria incapable of protein synthesis (Block and Blanchard 2021).

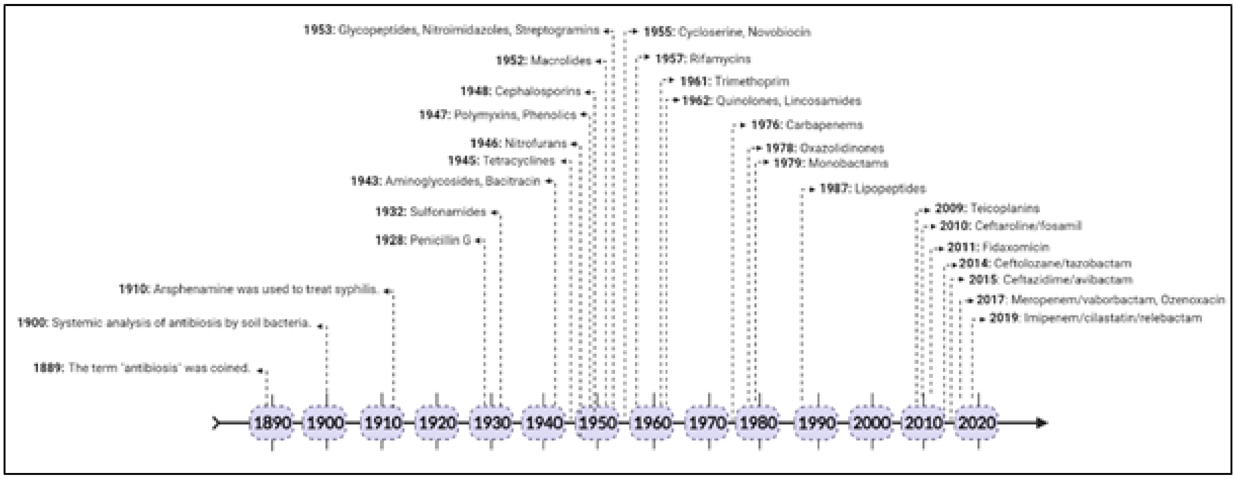
1953: Glycopeptides, Nitroimidazoles, Streptogramins
1955: Cycloserine, Novobiocin
1952: Macrolides
1957: Rifamycins
1961: Trimethoprim
1948: Cephalosporins
1962: Quinolones, Lincosamides
1947: Polymyxins, Phenolics
1976: Carbapenems
1946: Nitrofurans
1978: Oxazolidinones
1945: Tetracyclines
1979: Monobactams
1943: Aminoglycosides, Bacitracin
1987: Lipopeptides
1932: Sulfonamides
2009: Teicoplanins
2010: Ceftaroline/fosamil
1928: Penicillin G
2011: Fidaxomicin
2014: Ceftolozane/tazobactam
2015: Ceftazidime/avibactam
1910: Arsphenamine was used to treat syphilis.
2017: Meropenem/vaborbactam, Ozenoxacin
1900: Systemic analysis of antibiosis by soil bacteria.
2019: Imipenem/cilastatin/relebactam
1889: The term "antibiosis" was coined.
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Fig. 1: Timeline of the discovery of antibiotics.

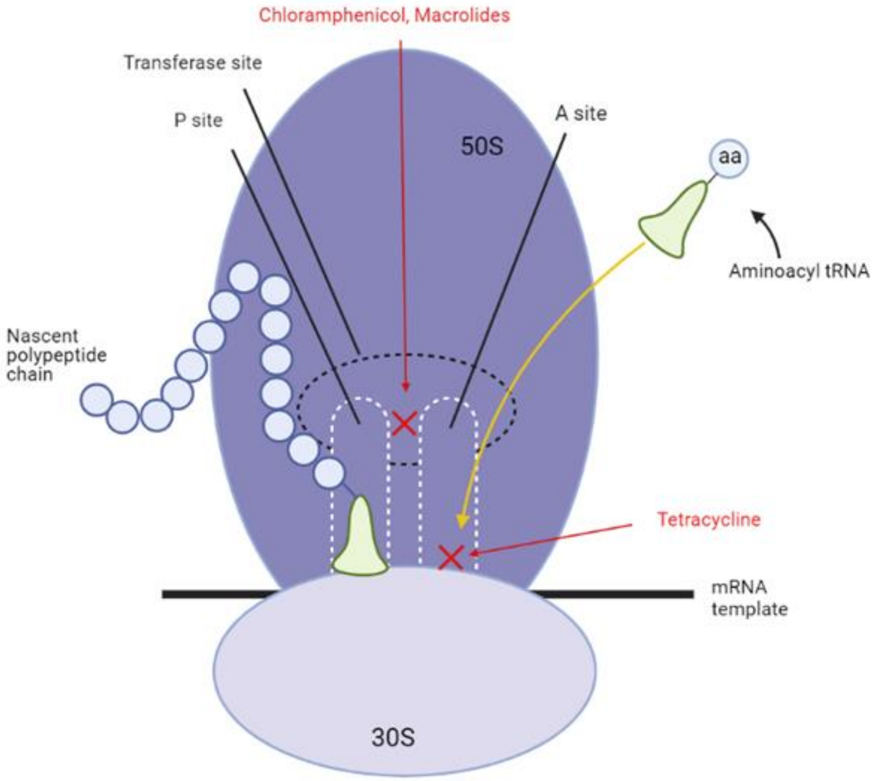

Fig. 2: Inhibition of bacterial protein synthesis by chloramphenicol, macrolides, and tetracyclines. mRNA attaches to the bacterial 30S ribosomal subunit. The nascent polypeptide chain is in the P (peptidyl) site. The aminoacyl tRNA carrying an amino acid (aa) moves into the A (acceptor) site, with base pairing between the mRNA and tRNA. Tetracyclines bind to the 30S subunit and inhibit protein synthesis by blocking tRNA binding to the A site. Chloramphenicol inhibits transpeptidation by binding to the 50S ribosomal subunit at the peptidyltransferase. Macrolides inhibit protein synthesis by binding reversibly to the 50S ribosomal subunits such that the nascent peptide chain temporarily residing at the A site fails to move to the P or donor site.

β-lactam antibiotics comprise penicillins, cephalosporins, carbapenems, and monobactams (Fig. 3). All contain a β-lactam ring and kill susceptible bacteria by suppressing the transpeptidase that triggers the last step in cell wall synthesis (MacDougall 2017). β-lactams inhibit bacterial cell wall, particularly peptidoglycan, synthesis. Peptidoglycans are the bacterial exoskeleton that strengthens the cells' integrity and shape and prevents bursting (Auer and Weibel 2017). These structures cross-link in the last stage of cell wall formation to produce peptidoglycan polymers via membrane -anchored

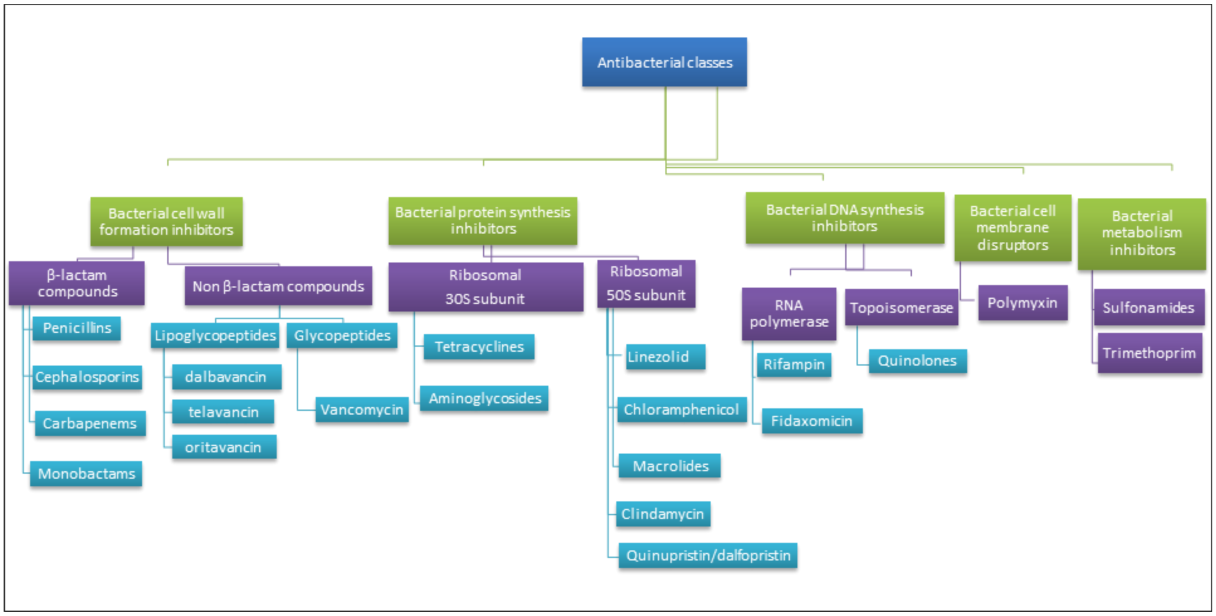

Fig. 3: Major classes of antibacterial compounds.

enzymes such as transpeptidases, carboxypeptidases, and endopeptidases, collectively known as penicillin-binding proteins (PBPs). Penicillin exerts antibacterial activities by inhibiting cell wall formation (Stepek et al. 2019). The drug's binding to the target organism's proteins inhibits PBPs, which are naturally occurring proteins in the cell wall. Once targeted, these proteins are inhibited from completing the peptidoglycan formation, which is essential to bacterial cell wall synthesis. This cell wall synthesis inhibition, combined with the ongoing cell wall degradation via the action of cell wall autolytic enzymes, finally causes bacterial cell lysis and death (Bazakis et al. 2022).

Cephalosporins are a large group of bactericidal β-lactam antibiotics used to treat Gram+ve and Gram-ve bacterial infections that act via β-lactam rings. After the attachment of the β-lactam rings to the PBP, normal activity is inhibited. Consequently, bacteria become incapable of synthesizing a cell wall and die eventually. *Staphylococcus* (*S.*) *aureus*, usually susceptible to cephalosporins, can become resistant by altering the PBPs' structure. Resistant *S. aureus* possesses a gene encoding a modified PBP, inhibiting the cephalosporin's β-lactam rings from deactivating the protein. Resistant bacteria through this mechanism are called methicillin-resistant *S. aureus* (MRSA). Another important resistance mechanism is producing β-lactamase, an enzyme that cleaves the β-lactam ring and inhibits it from attaching to the PBPs, such as peptidoglycan transpeptidase. Inhibitors of β-lactamase can be co-formulated with cephalosporins to improve their activity spectra, such as ceftazidime/avibactam and ceftolozane/tazobactam (Bui and Preuss 2021). Most bacteria have at least one PBP, which is the target for various β-lactam antimicrobials, such as cephalosporins, penicillins, carbapenems, and monobactams (Parsels et al. 2021).

Vancomycin is a non-β-lactam glycopeptide antimicrobial with bactericidal activity by preventing cell wall synthesis and is active against Gram+ve bacteria. It attaches to the D-alanyl-D-alanine (D-Ala-D-Ala), the C-terminal peptide structure of the peptidoglycan precursor in the bacterial cell wall, via hydrogen bonds. When the precursor interacts with vancomycin, transglycosylase cannot handle it, inhibiting cell wall synthesis. Afterward, replicating bacteria die due to a disrupted cell wall, making them susceptible to osmotic pressure (Mühlberg et al. 2020).

Polymyxins are a group of bactericidal drugs that disrupt the bacterial cell membrane. These are basic peptides with a molecular weight of about 1000 Da and act as amphipathic surfactants or cationic detergents. Polymyxins interact powerfully with phospholipids and interrupt cell membranes' integrity via binding to lipopolysaccharides and phospholipids in Gram-ve bacteria cell membranes. The underlying mechanism of action displaces divalent cations from membrane lipids' phosphate groups and destabilizes the cell membrane, causing leakage of the intracellular content and cell death (Ayoub Moubareck 2020).

Quinolones are broad-spectrum antimicrobials active against Gram +ve and Gram -ve bacteria. DNA gyrase is the primary target of quinolones, shown to catalyze the ATP-dependent production of negative supercoiled DNA. Maintaining DNA supercoiling is vital for the proper regulation of various biological processes. The resolution of DNA supercoiling is crucial to maintain DNA replication rates, and topoisomerases manage this resolution. Topoisomerases' activity is necessary and constitutes a prime antibiotic target (Bhagwat et al. 2019). Topoisomerases are necessary for plasmid replication and the regulation of phage recombination (Gutierrez et al. 2017). This discovery offered new tools to understand the detailed mode of quinolones' bactericidal action.

Fluoroquinolones are an essential broad-spectrum antimicrobial class. Nalidixic acid was the first described quinolone, showing a narrow spectrum of antibacterial activity. Changes in positions 1, 6, 7, and 8 of nalidixic acid's chemical structure have led to newer, more potent quinolones. Quinolones prevent DNA gyrase and topoisomerase IV, which are necessary for bacterial growth (Lapointe et al. 2021).

Chromosomal mutations often cause quinolone resistance by decreasing drug accumulation through reduced drug uptake or increased efflux. Resistance may also occur when quinolone resistance genes are exchanged on plasmids during bacterial conjugation. For example, the *qnr* gene encoding a pentapeptide, which inhibits quinolones' action on the DNA gyrase and topoisomerase IV, can be transported from one bacterium to another through the plasmid. Also, the *aac(6′)-Ib-cr* gene encodes an acetylase that modifies the amino group of fluoroquinolones' piperazine ring and is exchanged via the plasmid (Fàbrega et al. 2009).

Rifampin is a bactericidal antimicrobial that inhibits DNA-dependent RNA polymerase (RNAP). This inhibition occurs via blocking the elongating RNA at the 5′ end or reducing the RNAP affinity for short RNA strands. Rifampin inhibits the microbial RNAP and stops further RNA elongation (Suresh et al. 2021).

Sulfonamides are synthetic bacteriostatic drugs still used today to treat bacterial and other infections (Gulçin and Taslimi 2018). Sulfamethoxazole is a sulfonamide that inhibits folate synthesis inside microorganisms such as bacteria. It is *p*-aminobenzoic acid (PABA) competitor during dihydrofolate synthesis by inhibiting dihydrofolate synthase. Trimethoprim directly inhibits dihydrofolate reductase, inhibiting the synthesis of tetrahydrofolate (the active form of folate). Combining a sulfonamide and trimethoprim creates a synergistic anti-folate effect and inhibits the synthesis of tetrahydrofolate, a compound necessary for producing purines involved in DNA and protein synthesis. These drugs are bacteriostatic when used separately, while the sulfamethoxazole-trimethoprim combination blocks two steps in the bacterial synthesis of essential nucleic acids and proteins and is bactericidal (Kemnic and Coleman 2021).

Antibiotic Resistance (AR)

AR occurs when germs develop mechanisms to defeat the drugs that kill them, which means the bacteria continue to grow. AR is an ancient mechanism since most antibiotics currently used in humans are natural compounds of bacterial or fungal origins. *Streptomyces* are soil bacteria that are the source of most of the currently available antibiotics. Microorganisms have to fight against each other in their natural environment by secreting antimicrobial chemicals and have to become resistant to other antibiotics. Species naturally producing antibiotics are resistant to these substances to avoid self-toxicity (Meade et al. 2020).

Interestingly, multidrug-resistant (MDR) bacterial species and resistance genes to currently available antibiotics have also been purified from environmental and archeological specimens. β-lactamase encoding *blaOXA* genes date back several million years (Bush 2018). D'Costa and coworkers isolated resistance genes to β-lactams, tetracyclines, and glycopeptides from 30,000-year-old permafrost specimens (D'Costa et al. 2011). Kashuba and her colleagues also discovered several resistance genes in a *Staphylococcus* (*S.*) *hominis* genome from permafrost at Mammoth Mountain in Siberia, returning to the Middle Miocene. The recovered *S. hominis* had multiple genes associated with resistance to aminoglycosides, β-lactams, macrolides, lincosamide, and streptogramin B (Kashuba et al. 2017). 177 AR genes in 23 families, representing all resistance mechanisms, i.e., mutation, efflux, and antibiotic deactivation, were discovered in microbes isolated from the Mackay Glacier region (Van Goethem et al. 2018; Durand et al. 2019).

AR has become an issue of increasing concern to the medical community. According to the 2019 AR Threats Report of CDC, more than 2.8 million antimicrobial-resistant infections take place in the U.S. annually, killing more than 35,000 individuals. Annually, >2,000,000 North Americans get AR-associated infections, causing 23,000 deaths. In Europe, about 700,000 antibiotic-resistant cases culminate into over 33,000 deaths yearly (Cassini et al. 2019), costing over €1.5 billion (ECDC 2009). Despite a 36% rise in human use of antibiotics between 2000 and 2010 (Laxminarayan et al. 2016), around 20% of worldwide deaths are due to infectious diseases (Martens and Demain 2017). Unfortunately, nosocomial infections have become a principal cause of morbidity and mortality (Akova 2016), causing lengthier hospital stays and increased healthcare expenditures (McFee 2009). Unfortunately, the outlooks are more pessimistic. For example, a government-accredited study in the United Kingdom forecasts 10,000,000 annual deaths from antimicrobial-resistant infections by 2050 (O'Neill 2018).

Antibiotics are primarily applied in food-producing animals (FPAs) worldwide. These days, the effective production of FPAs is partly due to incorporating growth supplements (Price et al. 2005) and large-scale antibiotic use as growth promoters in livestock farming (Rather et al. 2017). For instance, 60% of the antibiotics sold to the U.S.A. food industry are also used as human therapeutics (Dodds 2017). The average worldwide annual antimicrobial consumption by animals in 2010 was estimated at 45 mg/kg, 48 mg/kg, and 172 mg/kg for cattle, chickens, and swine, respectively. Moreover, using antibiotics in FPAs is projected to increase by 2030 (Price et al. 2005). The widespread antibiotic use in FPAs contributes to selective pressure, favoring AR development and negatively impacting public health.

Antimicrobial-resistant pathogens can be transmitted to humans from the environment or food products and thus become a public health concern (Mohsin et al. 2017).

Pakistan is one of the top 10 animal-producing countries with modern intensive farming practices, and food animals have become crucial for food security. Unfortunately, like in many other countries, animal farming in Pakistan largely depends on consistent antibiotic use as growth promoters and therapies for bacterial diseases (Ur Rahman and Mohsin 2019). The overuse of antibiotics in animal production and the later land applications of manures lead to elevated AR levels in the soil environment. Understanding the fate of antibiotics and how AR genes spread from animal production systems to the soil is essential to minimize AR risks (Chaturvedi et al. 2021).

Veterinary drugs, especially antimicrobials, are one of the most critical issues related to animal feed production. The primary use of antibiotics in animals is to prevent and treat diseases and promote growth (Ahmad and Khan 2019). The application of antibiotics in animals may result in antibiotic residues in foodstuffs such as meat, milk, and egg. These remnants may cause several side effects, such as the spread of antibiotic-resistant bacteria, immunopathological consequences, allergy, mutagenicity, nephropathy (aminoglycosides), hepatotoxicity, reproductive diseases, bone marrow toxicity (chloramphenicol), and carcinogenicity (sulfamethazine, oxytetracycline). Because of these unwanted effects, it is vital to regulate the use of antibiotics. The most crucial adverse effect of antibiotic remnants is transferring antibiotic-resistant bacteria to humans. However, it is crucial not to forget the effect of AR on the animals, as infection by resistant bacteria leads to the loss of animals and flocks due to treatment failure (Bacanlı and Başaran 2019).

Bacteria must not necessarily be resistant to every antibiotic to be life-threatening, and resistance to even one antibiotic can be dangerous. For example, antibiotic-resistant infections necessitating second and third-line therapies can harm patients by causing severe side effects, such as organ failure and prolonged recovery, often lasting months. Many medical procedures depend on handling infections using antibiotics, including orthopedic surgeries, organ transplants, debulking cancer, and treating chronic diseases like asthma, diabetes, and rheumatoid arthritis. These infections usually have no treatment options (Durand et al. 2019).

New Antibiotics in the Last Two Decades

A small number of new antibiotic compounds have been developed since 2000, and none of them is effective against Gram-ve bacteria. These results indicate that new antibiotics targeting the gravest public health threats have not been introduced (World Health Organization 2019). Drugs developed since 2008 fall into four categories: lipoglycopeptides, β-lactam/ β-lactamase inhibitors, quinolones, and protein synthesis inhibitors (Bassetti and Righi 2015). The FDA-approved multi-target antibiotics, their target sites, and their structural classes are listed in Table 1.

Lipoglycopeptide Antibiotics

Modernized lipoglycopeptides are a subclass of glycopeptides distinguished by a lipophilic side chain attached to a glycopeptide moiety. These compounds include teicoplanin and its derivatives dalbavancin, telavancin, and oritavancin (Morphy et al. 2004). The FDA-approved telavancin was used in 2009 to medicate complicated skin and skin structure infections caused by multidrug-resistant *S. aureus* or other Gram+ve pathogens and in 2013 to medicate pneumonia caused by *S. aureus* and *Streptococcus pneumoniae*. The FDA approved the drug in 2014 to treat acute bacterial skin infections caused by pathogenic Gram+ve bacteria. The same year, dalbavancin was licensed for acute bacterial skin infections by antibiotic-resistant pathogenic Gram+ve microbes (Markham 2014). Teicoplanin and its derivatives block the cell wall peptidoglycan layer cross-linking by binding to the lipid II monomer's D-alanyl-D-alanine (D-Ala-D-Ala) terminal (Wetzel et al. 2021).

Oritavancin and telavancin maintain vancomycin's heptapeptide core, consisting of seven amino acids: five aromatics and two aliphatics. On the other hand, dalbavancin contains teicoplanin's cyclic heptapeptide core, consisting of seven aromatic amino acids. D-Ala-D-Ala binding prevents transpeptidase-mediated peptide cross-linking. Telavancin, oritavancin, and dalbavancin also block peptidoglycan construction via impairing transglycosylase-mediated glycan cross-linking. The lipophilic side chains of teicoplanin, oritavancin, and telavancin enhance their dimerization and peptidoglycan binding and may disrupt the bacterial membrane integrity as the third target of these compounds (Wetzel et al. 2021).

The dalbavancin's hydrophobic group enhances dimerization, and bacterial membrane disruption by dalbavancin has not been elucidated (Cheng et al. 2014). The second-generation lipoglycopeptides show better pharmacodynamic and pharmacokinetic characteristics than vancomycin (Das et al. 2017), and these are also less susceptible to resistance development by bacteria. Bacteria can avoid an antibiotic's action on a given target through genetic mutation. Because the second-generation lipoglycopeptides affect more than a single target, random mutations that protect each target are needed and extremely improbable statistically (Zhanel et al. 2010).

Modernized β-lactam Antibiotics

Ceftaroline/Fosamil and Cefiderocol*:* Cefiderocol was approved by the FDA in 2019 for urinary tract infections caused by multidrug-resistant Gram-ve bacteria. FDA also approved ceftaroline fosamil in 2010 to treat community-acquired bacterial pneumonia and acute bacterial skin infections caused by Gram-ve microbes (Moore et al. 2020). These are multi-target antibiotics developed by rational design and belong to the cephalosporin subclass,

Table 1: Structural classes and primary targets of FDA-approved antibiotics since 2008

Cell Wall Synthesis		DNA Replication and Transcription	Protein synthesis inhibitors
Lipoglycopeptides	β-lactam/β-lactamase inhibitors	Quinolones	
dalbavancin	cefiderocol	delafloxacin	eravacycline
oritavancin	ceftaroline fosamil	finafloxacin	plazomicin
telavancin	ceftazidime/avibactam	gatifloxacin	
	ceftolozane/tazobactam	moxifloxacin	
	imipenem/cilastatin/relebactam	ozenoxacin	
	meropenem/vaborbactam	fidaxomicin	

included under the β-lactam antibiotics (Sato and Yamawaki 2019). The cephem moiety of ceftaroline fosamil and cefiderocol is involved in the principal action to inhibit bacterial cell wall formation via irreversible peptidoglycan transpeptidase inhibition (Wetzel et al. 2021).

Ceftolozane/Tazobactam, Ceftazidime/Avibactam, Meropenem/Vaborbactam, and Imipenem/ Cilastatin/Relebactam: Four combinatorial β-lactam/β-lactamase inhibitor combinations were lately approved by the FDA. These combinations are ceftolozane/tazobactam (approved in 2014), ceftazidime/avibactam (approved in 2015), meropenem/vaborbactam (approved in 2017), and imipenem/cilastatin/relebactam (approved in 2019). These combinations are used for urinary tract infections, abdominal infections, and pneumonia (Papp-Wallace et al. 2020).

Modernized Quinolone Antibiotics

Fluoroquinolones: Finafloxacin (approved in 2014) and delafloxacin (approved in 2017) are fourth-generation fluoroquinolone antibiotics. These synthetic compounds have the same bicyclic nitrogen and keto-containing core structures. Quinolones inhibit one or both bacterial DNA gyrase and topoisomerase IV (Yamakawa et al. 2002). DNA gyrase modulates bacterial DNA replication and transcription by regulating the DNA's negative supercoiling, while topoisomerase IV mediates unlinking of post-replication daughter strands (Ashley et al. 2017).

First-generation quinolones (e.g., nalidixic acid) chiefly act on Gram-ve bacteria, while second-generation compounds (e.g., trovafloxacin, ciprofloxacin) exert activity on Gram-ve and some Gram+ve microbes. Third-generation quinolones (e.g., levofloxacin) exert an extended anti-Gram+ve spectrum (Dehbanipour et al. 2019) due to their selective topoisomerase IV inhibition, as opposed to DNA gyrase inhibition by first- and second-generation counterparts. The fourth-generation quinolones (e.g., delafloxacin, gatifloxacin, and moxifloxacin) inhibit DNA gyrase and topoisomerase IV. Hence, these are desirable for treating quinolone-resistant microbial diseases (Pham et al. 2019). Moxifloxacin, gatifloxacin, delafloxacin, and finafloxacin inhibit topoisomerase IV and DNA gyrase at equivalent rates (Saravolatz and Leggett 2003). Trovafloxacin and ciprofloxacin are eight and nineteen times more potent topoisomerase IV inhibitors than DNA gyrase (Wetzel et al. 2021).

The dual-targeting characteristic of fourth-generation quinolones renders them suitable for use against drug-resistant microbes since the simultaneous mutation at two sites of the target proteins is uncommon. Moreover, concurrent inhibition of two enzymes in the same pathway guarantees pathway inhibition (Wagenlehner et al. 2018).

Nonfluorinated Quinolones: Ozenoxacin was the first nonfluorinated quinolone approved in 2017 for topical application to treat impetigo caused by *S. aureus* or *Streptococcus pyogenes* (Wren et al. 2018). Ozenoxacin blocks DNA gyrase and topoisomerase IV, while other quinolones primarily block one enzyme. DNA gyrase and topoisomerase are necessary for bacterial DNA replication and transcription (Vila et al. 2019). Another feature of ozenoxacin is that it cannot be pumped out of the bacterial cell by efflux pumps. These features make the drug a good choice for fluoroquinolone-resistant bacterial infections (López et al. 2013). In addition, since ozenoxacin is not fluorinated, it raises fewer safety issues than fluorinated quinolones (Vila et al. 2019).

Fidaxomicin (lipiarmycin A3, clostomicin B1, tiacumicin B) is a narrow-spectrum antibiotic active against Gram+ve anaerobes, including *Clostridium* (Artsimovitch et al. 2012). It is a natural product isolated from several soil bacteria and contains a glycosylated 18-membered macrolactone ring. Fidaxomicin was marketed in 2011 for intestinal infections due to *C. difficile*. The drug also demonstrates *in vitro* antibacterial activity on resistant *S. aureus* and *Mycobacterium tuberculosis* and exerts antibacterial effects by blocking RNA polymerase (Brauer et al. 2022).

Fidaxomicin and rifampin are bacterial transcription inhibitors. However, the former acts at an earlier step in the transcription initiation pathway (Babakhani et al. 2014), specifically binding to the DNA template-RNA polymerase complex. Fidaxomicin inhibits the initial DNA strand separation that forms the open DNA template-RNA polymerase complex, which precedes messenger RNA synthesis by blocking the σ subunit (Gualtieri et al. 2006). Fidaxomicin has a limited spectrum of antimicrobial activity since it has a unique target site (Tiwari et al. 2010). *C. difficile* isolates resistant to rifampin or other antibiotics (cephalosporins, fluoroquinolones) do not exhibit cross-resistance to fidaxomicin (Zhanel et al. 2015). Fidaxomicin is the drug of choice for treating colitis caused by *C. difficile*, the third most common nosocomial infection in the United States, causing 29,000 annual deaths (McAlpine 2017).

Protein Synthesis Inhibitors

In the last five years, the U.S. Food and Drug Administration (FDA) and the European Medical Agency (EMA) have approved several new antibiotics with prominent anti-Gram-ve bacterial activity, such as plazomicin and eravacycline.

Plazomicin: Plazomicin, approved by the FDA in 2018, is an aminoglycoside that binds to the ribosomal 30S subunit of bacteria, leading to protein synthesis inhibition. Plazomicin displays antibacterial activity against aerobic Gram-ve bacteria, such as β-lactamase-producing and carbapenem-resistant Enterobacteriaceae, and bacteria with aminoglycoside-modifying enzymes (Shaeer et al. 2019). The most common adverse effects of plazomicin are reduced renal activity, diarrhea, hypertension, headache, nausea, and vomiting. Plazomicin may block neuromuscular activity and cause fetal injury in pregnant women and ototoxicity. There is limited efficacy and safety data about plazomicin; hence, the antibiotic is indicated for treating complicated urinary tract infections in adults with few or no treatment choices. If plazomicin is used in patients with severe renal impairment, reducing dosage and drug monitoring are necessary (Clark and Burgess 2020).

Eravacycline: Eravacycline, a fluorocycline that belongs to the tetracyclines, was approved by the FDA in 2018. It is a bacterial protein synthesis inhibitor active against multidrug-resistant organisms, such as *Acinetobacter*, methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*, and β-lactamase-producing and carbapenem-resistant Enterobacteriaceae. Eravacycline treats complicated intra-abdominal infections, and the most common adverse effects are infusion site reactions, nausea, vomiting, and diarrhea (Heaney et al. 2019).

Conclusion

Antibiotic resistance is an increasingly common occurrence around the globe, especially in developing countries, and bacteria have developed mechanisms to combat antibacterial product activity. Antibiotic usage must be better regulated locally and globally, including in developed countries. Along with global public awareness, there is a need to stop using over-the-counter antibiotics and educate prescribers to reduce antibiotic resistance.

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Chagas Disease

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INTRODUCTION

Chagas disease is caused by the protozoan parasite *Trypanosoma* (*T.*) *cruzi*, also known as American trypanosomiasis. The disease was discovered in rural Latin America in the 20th century (1909) and currently affects about 6 million people worldwide. It is transmitted to the final host with the help of a Triatomine bug. The bug defecates during the blood meal and transmit *T. cruzi* to the final host through stercorarian route. Transplantation or congenital blood transfusion can transmit *T. cruzi*. These bugs usually live in crevices on the roofs of houses which are normally active at night and hide during daytime. This parasitic and chronic infection is the cause of cardiac or digestive complications that leads to patient death (Monteiro et al. 2022). This disease is called a silent disease because the symptoms of this disease in affected people may not be shown too early. The case detection rate for this disease is estimated to be 10%, creating a barrier to treatment and transmission. Every year around 12,000 people die due to complications from Chagas disease, with only 1 in 10 persons being diagnosed. Of these affected people, only a few receive treatment. A decrease in cases has been seen from 1980 to 2010 when the cases reduced from 17 million to less than 6 million. This was made happen by interrupting the blood transfusion transmission (LE et al. 2020). Animals are also seen to serve as carriers including rabbits, cats, and rodents. Dogs and cats are recognized as reservoirs of parasites (Gürtler et al. 2007).

The Chagas disease affect both humans and animals and has a large number of cases every year. Considering the significance of protozoa, this chapter briefly describes the parasite morphology, prevalence, life cycle, treatment, and various control strategies to reduce the infection rate.

Morphology

T. cruzi consists of a single tubular mitochondrion and has features like mammalian cells i.e., cristae and the presence of DNA (WHO 2002). In the mitochondria, the Kinetoplast consists of a DNA network comprising 22-25% of the total parasite DNA called kinetoplast DNA (Macedo et al 2010). *T. cruzi* whose developmental stages lack kinetoplast form can't perform a normal cycle. The shape and size of kinetoplasts fluctuate during different stages of development. Metabolic and structural changes in parasitic life cycle occurs due to multiple biological changes that make infection visible (Vickerman 1985).

The vector of disease is a triatomine bug belonging to the family Reduviidae, subfamily Triatominae and order Hemiptera (Corrêa et al. 1998). The protozoan parasite consists of three evolutive forms i.e., amastigotes, epimastigotes and trypomastigotes that can be identified with optic microscopy, by keeping in view the kinetoplast position in comparison to the nucleus and with the help of flagellum visibility (Hoare 1972).

T. cruzi contains an intracellular form that is the amastigote form seen in the tissues of the vertebral host. There is a lack of exterior flagellum and undulating membrane. Its measurement is 4.0 µm in diameter. The binary division takes place every 12 hours and trypomastigote is formed roughly 10-12 hours before rupturing of the cell. The amastigote stage forms and can infect newly formed cells in circulation (Ley et al. 1988).

The epimastigotes form is present in the opossum anal glands and the triatomine digestive tract. A kinetoplast and the flagellum are present before the nucleus, a poorly developed membrane is also present, measuring up to 40µm, and consisting of freely floating flagellum. In general, the nucleolus at the nucleus's center position is present (López-Velázquez et al. 2005). It shows movement and presents highly replicative activity. The binary division also seen, forming rosacea. The temperature of 37 ^{0}C severely affects the evolutive form, while the range of 20-28 ^{0}C shows a better development. (Kleisen et al. 1976; Pollard et al. 1990; Florencio-Martínez et al. 2010).

In trypomastigotes, the ability to replicate is lacking, but submembrane microtubules are present that perform many functions i.e., cell differentiation, motility and tissue

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migration and are considered extrinsic infectious forms. There are two kinds of trypomastigotes found in the cells of invertebrates and vertebrate hosts. Metamorphic trypomastigotes are found at the end of the intestine or may be present in Malpighian tubules. Morphologically, it is similar in appearance to narrow form of the blood trypomastigotes, up to 17µm in size. A shorter flagellum appears near the basket-shaped kinetoplast, and a narrower wavy membrane appears along it. Blood and other body fluids may have trypomastigotes in the blood. The size of the wave film ranges from 12 to 20µm (Logan and Menko 2019).

Prevalence

Initially, Chagas disease was observed in Latin America in the early 16th century, affecting European people (Miles 2004). In 1950, the first vector-borne Chagas disease was reported in the United States. Immigration also contributes to the disease increase from endemic to non-native countries (Develoux et al. 2010).

From 1994 to 1998, donor screening was conducted in Los Angeles and Miami to find donors who had been born in or had previously travelled to Chagas disease-endemic countries (Leiby et al. 2002). Of these, 7.3% of the donors from Los Angeles and 14.3% from Miami were identified as high-risk donors. With radioimmunoprecipitation assay (RIPA) validation and enzyme immunoassay (EIA) screening, *T. cruzi* was detected in 7,500 donors in Los Angeles and 9,000 donors in Miami. The Los Angeles seroprevalence rate increased each year dramatically during the study period. On average, 25,000 to 30,000 donors were screened for seropositivity. Seroprevalence was highest in places with large populations of immigrants from endemic countries. In 2009, it was estimated that up to 300,000 US immigrants had chronic *T. cruzi* infection, almost 30,000 to 45,000 cases of Chagas cardiomyopathy and up to 300 congenital infections annually based on immigration and seroprevalence data in endemic countries (Bern et al. 2011).

Europe has reported isolated cases of Chagas disease since 1980s (Pehrson et al. 1981). According to the International Organization for Migration, the migration to Europe especially southern Europe has increased since 2000. According to census data from countries where Chagas is endemic, there were an estimated 4,83,074 legal immigrants from Latin America residing in European countries in 2005. 14,010 (2.9%) of them were considered contaminated and 2,803 acquired medical attention (Schmunis and Yadon 2010).

Life Cycle

Life cycle is completed in two different hosts i.e., vertebrate host include human and invertebrate host include reduviid or bed bug (*Triatoma infestans*). The infective metacyclic trypomastigote stage is injected in to vertebrate host through faeces of vector during the act of biting. The faecal material is being rubbed on the wound created during blood meal. Metacyclic trypomastigote is invasive in nature and penetrate various cells at the site of wound and transformed in to the amastigote stage. This stage start multiplying by binary fission inside the cells of infected tissues. Afterward, this stage gets converted to trypomastigote stage which is then released from the infected tissues after bursting the cell and enter in the blood stream. Trypomastigote can be converted back to amastigote stage and infect other tissues in the vertebrate host. When vector takes a 2nd blood meal from same host, it ingested the trypomastigote stage with blood. Trypomastigote stage transformed to epimastigote stage in the midgut of vector followed by the multiplication of epimastigote through binary fission. It is then transformed to trypomastigote stage in the hind gut of vector which ultimately injected again in the vertebrate host. Fig. 1 illustrate various life cycle stages of *T. cruzi* in vertebrate and invertebrate hosts (Rassi and Marin-Neto 2010).

Clinical Signs

Acute Infection

The parasite needs 1-2 weeks for incubation. The intense phase lasts almost 12 weeks. Patients with this parasite usually are asymptomatic. Only nonspecific symptoms are visible, like fever, headache, diarrhea, and vomiting. They do not need any medical care (Prata 2001). Only 1% of people show severe conditions like myocarditis and pericardial effusion. Acute disease has a danger of mortality rate of upto 0.2–0.5%. Under a microscope, trypomastigotes appear like a smear of a bluffy coat. In some patients, blister forms at the injection site which is usually referred as chagoma. Lesions may also be formed. The occurrence of disease is more in older people than the children older than two years of age. Orally transmitted *T. cruzi* infection causes more mortality and morbidity (Carod-Artal 2007). During an outbreak in Venezuela almost 75% of infected people were symptomatic, 59% individuals were seen having ECG abnormalities, among them 20% were treated, and there were 1% death rate (Alarcón de Noya et al. 2010).

Congenital Infection

Most contaminated neonates are asymptomatic or show minimal symptoms, while a small percentage develop a severe life-threatening illness (Bern et al. 2009). The clinical signs of symptomatic congenital Chagas disease include hepatosplenomegaly, anemia and thrombocytopenia (Bittencourt et al. 1981). Meningoencephalitis, gastrointestinal mega syndromes, anasarca, pneumonitis, and/or respiratory transportation are among the severe neonatal complications that may occur. According to published data, newborns with infections typically die between 5% to 20% more than infants without infections (Torrico et al. 2004).

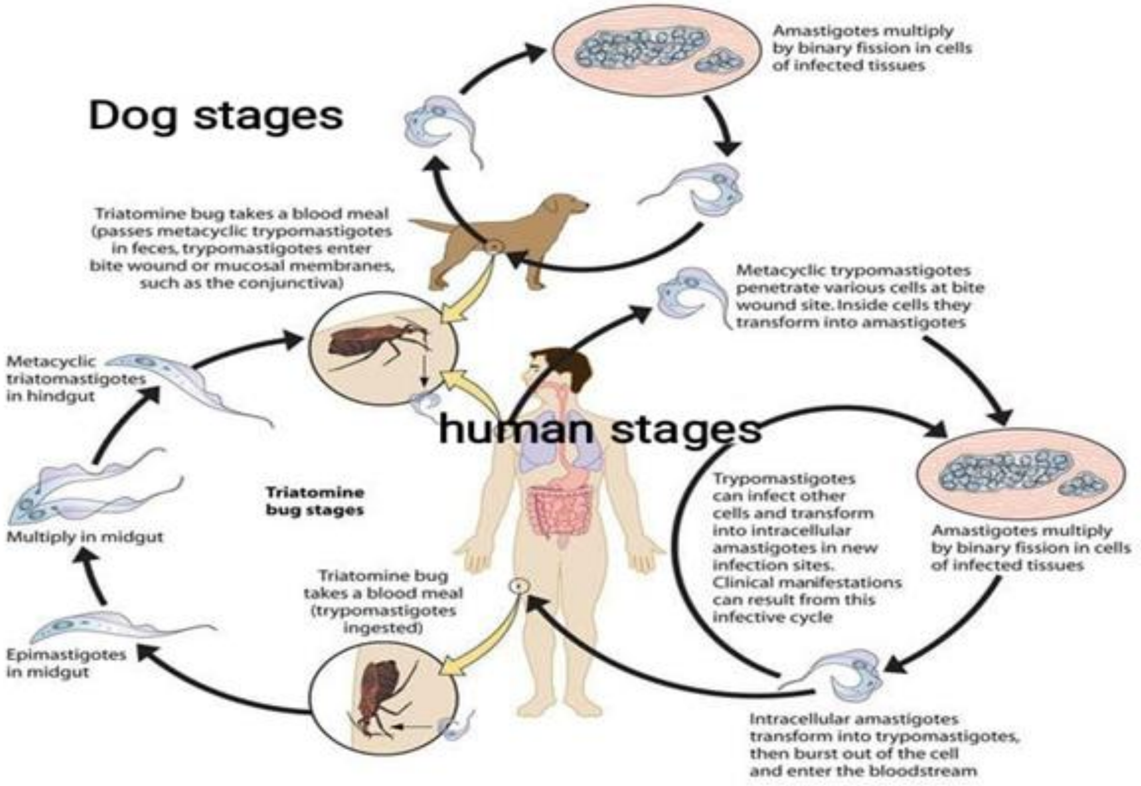
Dog stages
Amastigotes multiply by binary fission in cells of infected tissues
Triatomine bug takes a blood meal (passes metacyclic trypomastigotes in feces, trypomastigotes enter bite wound or mucosal membranes, such as the conjunctiva)
Metacyclic trypomastigotes penetrate various cells at bite wound site. Inside cells they transform into amastigotes
Metacyclic triatomastigotes in hindgut
human stages
Triatomine bug stages
Multiply in midgut
Trypomastigotes can infect other cells and transform into intracellular amastigotes in new infection sites. Clinical manifestations can result from this infective cycle
Amastigotes multiply by binary fission in cells of infected tissues
Triatomine bug takes a blood meal (trypomastigotes ingested)
Epimastigotes in midgut
Intracellular amastigotes transform into trypomastigotes, then burst out of the cell and enter the bloodstream

Fig. 1: Life cycle stages of *T. cruzi* in vertebrate and invertebrate hosts

Chronic Infection

After 8-12 weeks of infection, when parasitemia levels are no longer visible under a microscope, the person enters the frightful period of *T. cruzi* contamination in the nonappearance of successful etiological treatment. People with terrible *T. cruzi* infection can indirectly transmit the parasite to other individuals through blood components, organ contribution and congenital transmission despite the lack of minute parasites in the peripheral blood (WHO 2002).

Diagnosis

Depending on the stage of the disease and the status of the patient suitable diagnostic test for *T. cruzi* infection can be performed.

Diagnosis of Acute Infection

Motile trypomastigotes can be found during the acute phase under a microscope using the latest anticoagulated blood preparations or buffy coat. Parasites can also be observed under a microscope using Giemsa stain or other dyes on blood smears. While relatively sensitive during the acute phase, hemoculture in one of the standard parasite mediums taking two to four weeks to show reproduction. Within 90 days of infection, even without therapy, the parasitemia level declines and becomes microscopically undetectable during the chronic phase. PCR can be performed to detect the infection in patients who got the infection unintentionally or received an infected organ (Wegner and Rohwedder 1972).

Diagnosis of Congenital Infection

Similar diagnostic procedures are used for congenital Chagas' disease, which manifests as an acute *T. cruzi* infection in infancy. Fresh blood testing is less sensitive than the concentration methods. The procedure most frequently used in medical facilities in Latin America is the microhematocrit technique. With the help of light microscopy buffy coat layer is surveyed (Feilij et al. 1983). After delivery, parasitemia levels rise which peak up to 30 days or more. Repeated sampling within the first few months after delivery increases the sensitivity (Mora et al. 2005). Although the process is uncommon, but the molecular methods can identify congenital contaminants advance in life (Duffy et al. 2009). In developed nations like Latin America, PCR is the selected procedure for the timely identification of congenital disease. After nine months of age, when the transmitted maternal antibody has vanished, and the congenital infection has transitioned into the chronic phase, conventional IgG serology is advised for infants not identified at birth (Carlier and Truyens 2010).

Diagnosis of Chronic infection

Two serological examinations built on several procedures, such as immunoblotting, IFA and ELISA, are employed to boost the validity of the result because no single analysis has sufficient sensitivity and distinction to be relied upon lonely (WHO 2002).

Inevitably, some people subjected to two different tests whose serological results are inconsistent may require additional testing to determine their infection status. Because findings from less sensitive tests may yield negative results, specimens with the positive outcome but low antibody titers are probably disposed of the discrepancy. (Umezawa et al. 1999; Sosa-Estani et al. 2008). Trypomastigote-eliminated antigen immunoblot (TESA-blot) is another technique used to diagnose the chronic infection, but it does not have better susceptibility and may not be able to accurately identify the infection (Leiby et al. 2000). FDA has approved several ELISA kits for diagnostic use. Instead of relying on internal outcomes for which there are no conducting facts, it is preferable to utilize an assay with validation data (Bern et al. 2008).

Utility of PCR for Diagnosis

Accurate method for diagnosing acute stage and early congenital Chagas' illness, as well as checking for critical *T. cruzi* contamination in recipients of spoiled organs or after unintentional exhibition, is using PCR method (Chin-Hong et al. 2011). PCR assays typically produce positive results before the appearance of circulating trypomastigotes on peripheral blood smears (Schijman et al. 2000). The immunosuppressed host with *T. cruzi* contamination can be tested for reactivation using quantitative PCR techniques. An untimely and tactful predictor of reactivation in these patients is quantitative PCR testing, which shows growing parasite levels over time. Conventional PCR results in these patients do not verify reactivation (Diez et al. 2007). PCR is utilized in long-term *T. cruzi* contamination as an investigation apparatus, but typically it is not an adequate diagnostic trial. Even though a percentage of patients will have positive PCR results, the sensitivity varies greatly depending on the aspect of the residence being examined and PCR primers and techniques. For these reasons, negative PCR findings do not prove the absence of infection. (Basquiera et al. 2003).

Treatment

Only benznidazole and nifurtimox have been shown to effectively treat Chagas disease. Other medications besides these can be employed, but only by the inquiry procedure. A nitrofuran called nifurtimox disrupts the metabolism of carbohydrates in *T. cruzi* by preventing the formation of pyruvic acid. Between 30 % to 70% of patients experience side symptoms relating to the digestive system. Anorexia is included, which causes loss of weight, motion sickness, nausea and disconcertment in the abdomen. The symptoms of neurological poisoning, such as irritation, sleeplessness, confusion, and, less frequently, tremors, are also very typical. Polyneuropathy, peripheral neuritis and paresthesia are uncommon but have more severe adverse effects. The peripheral neuropathy, dependent on dose, develops late in therapy. Infants receive higher doses than older children, while children generally have better medication tolerance. A nitroimidazole derivative called benzmidazole is thought to be more trypanocidal than nifurtimox. The most common dermatological adverse effects are photosensitization-related rashes, which rarely proceed to exfoliative dermatitis. Dermatitis that is severe, exfoliative, or connected to a fever and lymphadenopathy should cause the medicine to be stopped right away. Peripheral neuropathy is dependent on dose, typically develops late in therapy and is a sign that treatment should be stopped immediately. This is reversible, but it may take months to determine. Beginning nine to ten days following the start of the analysis, patients should be checked for dermatological side effects (de Andrade et al. 1996).

Treatment of Acute and Congenital infection

Both medications lessen the simplicity of the signs, shorten the distance course, and shorten the period of apparent parasitemia in critical and early congenital Chagas disease. Nifurtimox was used in the initial anti-trypanosomal medication trials in the 1960s and 1970s on individuals with acute Chagas disease. 81% of patients who received acute phase treatment had serological cure verified at the 12-month follow-up (Bern et al. 2011).

Treatment of Chronic *T. cruzi* Infection

Antiparasitic drugs were thought to be only helpful during the acute phase, which includes early congenital infection. In contrast, two placebo-controlled studies were conducted in the 1990s on children with persistent *T. cruzi* infection and benzmidazole showed a cure rate of 60% (Gascón et al.2007).

Prevention

To prevent from chagas disease following precautionary measures must be taken:

- Using of insect powder and spray gun
- Have proper house cleanliness.
- Must follow different preventive measures such as transportation of food, consumption, and storage.
- Conduct seminars to provide awareness about this disease and how can we prevent it.
- Testing of organ, tissue or cell donors and receivers
- Do proper diagnosis and treatment of people with disease. specially women and children.

- Screening of infected mothers and newborn (Abad-Franch et al. 2010).

Conclusion

Chagas disease is caused by the protozoan parasite *T. cruzi*. Emigration of people from endemic to non-endemic region played a vital role in disease spread, which is heavily dependent on globalization. Lack of proper treatment and screening of infected people are significant causes of the spread of Chagas disease via non-vector routes of mother-to-child transmission and affected blood transmission. Despite various financial and political constraints, Chagas disease has been controlled through the invention of different medical and preventive techniques, social improvements, and transmission control. Comprehensive efforts are needed to engage immigrants and train healthcare providers to prevent the spread of disease.

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18

Veterinary Pharmacist: Standing at the Bridge for One Health Medicine

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INTRODUCTION

One heath approach is a collaboration between professionals in human and veterinary sector to treat the zoonotic diseases. The concept of "One Health" was not widely accepted until now. Through their functions as service animals, food producers, study subjects, and disease vectors, animals had a reciprocal effect on human health, therefore keeping pets and other animals healthy ultimately benefited the people. A more recent development is the convergence on an ecosystem health approach, which acknowledges the importance of considering environmental elements in relation to human and animal health and the interdependence of human, animal and environmental well-being (Gyles 2016).

The One Health Medicine Initiative has been active since 2006 and is doing excellent work in the United States and around the world by supporting One Health through teaching, lobbying, and therapy. The mission of this programme is to encourage the use of an interdisciplinary, cross-sectoral strategy for reducing health risks caused by human, animal, and environmental interactions. The recent occurrence of zoonotic illnesses like SARS and Ebola, as well as the potential for a pandemic of influenza, have served to emphasize the global significance of One Health medicine and an ecosystem approach (Gyles 2016).

Establishment of Veterinary Pharmacy Specialty

In the United States, veterinary pharmacy is a relatively new specialty. About 50 years ago, reported literature highlighted first time the pharmacist's involvement in veterinary medicine and veterinary biological. Historically, the functions of veterinarians and pharmacists have complemented one another (Ceresia et al. 2009). The pharmacist's job was to manufacture and dispense pharmaceuticals for animals, while the veterinarian's role was to diagnose and recommend treatment. Historically, veterinary colleges have hired pharmacists who have specialization in animal health and are responsible for the selection of medications, as well as the management of stock and formularies. Pharmacists are the only health care providers who are required by the society to treat patients of all species, despite the fact that veterinary pharmacotherapy is rarely taught in pharmacy schools (Ceresia et al. 2009). According to scenario, the National Association of Boards of Pharmacy passed a resolution in 2014 which emphasized the need of availability of veterinary pharmacotherapy education at pharmacy institutes in collaboration with institutes of veterinary medicine to enhance the competency of pharmacists in dispensing medications for veterinary patients (Emm 2020).

Veterinary pharmacists, like other pharmacists in the United States, acquired their education from a programme accredited through the Accreditation Council for Pharmacy Education (ACPE). However, unlike a human pharmacist, veterinary pharmacist often chooses to gain additional training through a post-doctoral experience or a position in veterinary medicine. Although an increasing number of pharmacy schools are beginning to offer electives in veterinary pharmacy and these are still rather uncommon (Kristine et al 2020). The American Society of Health-System Pharmacists (ASHP) Residency Directory has 1456 post-graduate year 1 (PGY-1) pharmacy residencies as of June 16, 2020, with only five PGY-1 clinical residencies in veterinary pharmacy. The residencies in the clinical veterinary pharmacy is currently available at veterinary schools of Purdue University, North Carolina State University, Texas A&M University, the University of Wisconsin and the University of California, Davis. When it comes to train the pharmacists, University of California, Davis residency position is unique because it is a co-funded programme by the Food Animal Residue Avoidance Databank and has a focus on preparing the residents to be competent in food animal pharmacotherapy and relevant litigious issues. After completing their residency programmes, most veterinary pharmacists work in either a veterinary teaching hospital, an academic setting, in a hospital pharmacy, a compounding pharmacy, or in a community pharmacy (Emm 2020).

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Organizations of Veterinary Pharmacy

The Society of Veterinary Hospital Pharmacists (SVHP) is an organization responsible for licensing of veterinary pharmacists and has 153 members in comparison to an estimated 64,000 members of the American Pharmacists Association. About half of SVHP's membership works in veterinary colleges of various universities. The American College of Veterinary Pharmacists (ACVP) is another association of veterinary pharmacy which has 182 pharmacists with 468 student members. Because of their expertise in compounding of medicines, these members offer both human and veterinary pharmacy care (Kristine et al. 2020). Only 32 veterinary pharmacists in the United States have completed the requirements for board certification and passed the 5-part examination given by the International College of Veterinary Pharmacy (DICVP) to demonstrate their masters of veterinary pharmacotherapy. Although, veterinary pharmacists provide health care to a number of species, together with exotic animals, companion animals, equine and the food animals, there are no other board-certified veterinary pharmacists for treating the specific species except the members of DICVP (Kristine et al. 2020).

Role of Veterinary Pharmacist in One Health Medicine

The CDC's One Health effort, aim is “to attain optimum health outcomes by connecting the people, animals, plants, and their shared environment” which highlights the significance of veterinary pharmacists. The new coronavirus that caused a global pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a tragic but illustrative example of the significance of One Health Medicine (Emm 2020). The SARS-CoV-2, first described in late 2019 in Wuhan, China, has jumped from species most likely from bats, and now it is infecting humans and transmitting from person to person. The possible transmission of diseases between humans and animals was properly detected, when a tiger in the Bronx Zoo tested for coronavirus illness (COVID-19) and it was positive under a care of zookeeper who was afflicted with the virus. Since then, the fecal samples of an additional four tigers and three lions have been found to be infected with the COVID-19 virus (Kim et al. 2020, Huang et al. 2020).

As the world grapples with cross-species transmission issues, infection diagnosis, and guidelines for interacting with household pets, a health practitioner trained to provide treatment for both human and nonhuman species is a wonderful advantage. A research highlighting this gap indicated that veterinarians wanted more interprofessional engagement with pharmacists in order to better understand the zoonotic illnesses. With some household pets testing positive for COVID-19, veterinary pharmacists are also retrieving a lot of questions from pet owners about the medications they are giving to their animals. Because of their specialized training, they realized that felids were coronavirus reservoirs prior to the pandemic, therefore their vulnerability to COVID-19 should come with no surprise (Root et al. 2013, Yoo and Yoo 2020). Despite the fact that canines are not generally thought to be at high risk of contracting coronaviruses since virus do not multiply very rapidly, more research has been required to determine the risk after a small number of canines tested positive for antibodies. Particularly in the context of community pharmacies, veterinary pharmacists are in a prime position to engage in two-way communication with clients, educating them on the potential impacts of COVID-19 on their pets and the ways in which they can mitigate these risks (Yoo and Yoo 2020).

Stewardship of the human food supply also includes the work of veterinary pharmacists, who ensured the absence of medicines in animal food and their meat or the presence of medicine should be below the level of federal threshold for pharmaceuticals in food. The National Institutes of Health hires veterinary pharmacists to ensure that laboratory animals are receiving a best pharmaceutical care. Veterinary pharmacists also have engagement in government settings to monitor and prevent the spread of animal-vector borne diseases that pose a risk to human health (such as leptospirosis, tick borne diseases, cat scratch fever, West Nile virus or rabies)

Involvement of Veterinary Pharmacist in Drug Discovery and Development

According to the International Conference on Harmonization (ICH), the first step in medication development in the United States is to conduct chronic toxicological testing on rodent and non-rodent animals, typically healthy rats and beagle dogs (Emm 2020). Animal experiments to determine efficacy were crucially significant during COVID-19 due to the need for viable therapy choices and the contradictory nature of the available evidence. After discovering that COVID-19 replicates in the soft palate, tonsils and nasal turbinate of ferrets, the specie was proposed as a natural animal model for the virus (Seah and Agrawal 2020; Daly 2020). The coronaviruses are known to affect a broad variety of animals, including exotic and domestic felines. During a pandemic, when information is scarce, natural animal models can be invaluable in determining the clinical application of innovative medicines by evaluating their efficacy in parallel. Data from pharmaceutical firms is available to help veterinarians to treat their patients as, under ICH guidance, medications looking for FDA approval undergo pharmacokinetic research in animal models. In 2018, the Kansas State University and the University of Missouri, Kansas City established a joint initiative called 1 Data to address this gap in the market and has been serving as a central repository to provide information on human and animal health. For the purpose of accelerating the development of medications for both humans and animals, as well as answering critical concerns that arise during the zoonotic outbreaks of COVID-19, this partnership aims to obtain animal and human data from a wide range of sources (Emm 2020).

Veterinarians treating animals could benefit from the knowledge of a veterinary pharmacist who is familiar with where to discover relevant animal pharmacokinetic data and its potential implications for public health. The FDA Emergency use authorization (EUA) of certain medications, including remdesivir, the best treatment of COVID-19, is also being tested against the Ebola virus before it was given the EUA. This drug has also shown promising results against other coronaviruses in animal studies, including Middle Eastern respiratory disease and severe acute respiratory syndrome (Ison et al. 2020). The EUA allowed doctors to start using a medicine in humans despite having limited data from clinical trials and animal research. Veterinary pharmacists are a valuable resource due to their ability to generalize from studies conducted on animals to human therapeutic practice. Due to a total lack of N-acetyl transferase in canid species, experimental drugs requiring acetylation during phase II metabolism are predicted to exhibit high toxicity in research dogs. An invaluable contribution that a veterinary pharmacist may make to a pharmaceutical company in creating a medicine would be to explain that toxicity has happened due to changes in metabolism, rather than pharmacological failure (Mealey 2019).

Role of Pharmacist in Climate Changes

People's health has been significantly impacted by anthropogenic global warming because of the increased severity and frequency of extreme weather measures and the resulting increase in air pollution. More than a quarter of all deaths around the world are attributed to cardiovascular disease, stroke, lung cancer, and chronic respiratory disease, all of which are exacerbated by greenhouse gas (GHG) emissions. The World Health Organization forecasts that between 2030 and 2050, this climate change will cause a further 250,000 fatalities annually as a result of hunger, malaria, diarrhoea, and heat stress. Annually, air pollution causes about 20,000 early deaths in Canada. It is particularly challenging for children, the elderly, and people with chronic illnesses to cope with life. The sector responsible for providing health care services is a substantial source of greenhouse gases. Canada's health care system contributes 4.6% to national GHG emissions, which is on par with the UK's (3%-4%), Australia's (7%), and the US's (9%-10%). Public hospitals (22%), pharmaceuticals (21%), and medical services (13%) are all significant contributors to greenhouse gas emissions in Canada's health care sector. Three-quarters of all greenhouse gas emissions from the healthcare sector come from drugs, both legally and illegally obtained (Roy 2021). About, 90 percent of these pollutants originate from upstream economic activity like producing energy or pharmaceuticals or operating transportation systems. Manufacturing, sourcing, delivery, disposal (through incineration), packaging, and use contribute to the GHG emissions associated with pharmaceuticals during the course of a drug's life cycle (Roy 2021). The carbon footprint of the UK National Health Service (NHS) has been significantly increased by the use of metered-dose inhalers (MDIs) and gaseous anaesthetics (2% and 4%, respectively). In comparison to carbon dioxide, the hydrofluoroalkane propellants used in MDIs are more hazardous and damage the environment by 3350 times more than CO_2. It has been calculated using data from the United Kingdom that the CO_2 emissions produced by a single patient's inhalers over the course of a year (12 controller inhalers at 19 kg CO_2 equivalent each and 3 salbutamol inhalers at 28 kg CO_2 equivalent each) are the same as those produced by driving a car up to 3,200 kilometers. Soft mist inhalers (e.g., Respimat) and dry powder inhalers (DPIs) emit less than 1 kg of CO_2 equivalent per inhaler, while these emissions are between 10 and 37 times higher. The anaesthetic gases responsible for greenhouse gas emissions include desflurane, which is 2540 times more harmful than CO_2, and nitrous oxide, which is less hazardous than desflurane but nevertheless used in large quantities across the healthcare industry. When compared to desflurane, the greenhouse gas emission profile for sevoflurane is seven times more favorable (Singleton et al. 2018).

In addition, medications are ingested and have detrimental effects on human health, human fetuses, and wildlife after entering water systems via drug production effluent, human excrement, and incorrect disposal. The global impact of pharmaceutical GHGs can be mitigated through measures such as reducing overall usage (through rational prescribing, deprescribing, and not storing), selecting less carbon-intensive goods (using DPIs), and ensuring correct disposal through incineration. When it comes to energy efficiency, transportation, and purchasing practices, the NHS is ahead of the curve. The carbon footprint of health and social services has decreased by 19% between 2007 and 2017 due to their efforts to cut carbon emissions. Key initiatives proposed by the health care sector to support a healthy world include leadership committed to environmental health, an immediate reduction in GHG emissions, and the procurement of sustainable products and resources, especially pharmaceuticals. In addition, the World Health Organization promotes the use of alternative energy sources, public transit, and active transportation methods like bicycling and walking as alternatives to automobile use to lessen the impact on the environment (Roy 2021).

Pharmacists, as highly respected members of the medical community and as potential movement leaders, are in a prime position to advance environmental protection. Large pharmaceutical carbon footprints highlighted the importance of pharmacists. For patient health and safety across the world, pharmacists can take the following steps.

1. Replace MDIs with DPIs or soft-mist inhalers if medically feasible. The National Health Service (NHS) has included a carbon footprint consideration into an asthma patient choice assistance for inhalers. In addition, teach patients how to effectively use their inhalers to cut down on waste (Roy 2021).
2. Improve the medication efficiency through rational and thoughtful prescribing. It has been estimated that 202 tonnes of greenhouse gas emissions may be saved if medicines are being used according to the recommendations of the National Institute for Health and Care Excellence guideline (Roy 2021).
3. Promote the use of inhaled anaesthetics that produce less carbon dioxide, such as sevoflurane instead of desflurane when necessary, and limit the overuse of nitrous oxide (Roy 2021).
4. Teach people under your supervision and in other fields how to safely dispose of medications, especially MDIs (i.e., return to the pharmacy for incineration) (Roy 2021).
5. Spread knowledge about health care sustainability to patients and medical staff.
6. Urge top-level administrators, policymakers, and medical and environmental groups to make sustainable healthcare a top priority (Roy 2021).
7. Create a community environmental group. Health Care without Harm (https://noharm.org/), Global Green and Healthy Hospitals (https://www.greenhospitals.net/), Safer Pharma (https://saferpharma.org/), and My Green Doctor (https://www.mygreendoctor.org/) are just few of the online resources available to help with this initiative. All over Canada, a number of hospitals and other medical facilities are participating in these environmental friendly programmes (Roy 2021).
8. Evaluate current habits to observe where you can make changes, such as using a bike or walking to work, holding virtual meetings with patients or coworkers, or reducing your paper use and switching to digital journal subscriptions (Roy 2021).

Conclusion

In the future, an increase in the number of qualified veterinary pharmacists may result in a workforce prepared to respond to one health concerns. Veterinary pharmacists are an underutilized resource and should cooperate with industrial and academic institutions to create treatment options and answer all inquiries about zoonotic diseases. More veterinary pharmacy residencies are required to address this need in healthcare system. Veterinary pharmacists can help answer problems about zoonotic disease by recognizing species differences, analyzing drug repurposing, collaborating in therapeutic research, and can address climate concern.

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Pathogenesis of Gizzard Erosion and Ulcerations and Comprehensive Control Strategy in Poultry

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INTRODUCTION

Ulceration in stomach has constantly been reported in poultry and livestock. It usually causes persistent diarrhea and poor absorption in animals, which leads to economic loss (Li et al. 2020, Wang et al 2021). Gizzard erosion and ulceration syndrome (GEU) was firstly reported in chicken in 1930s. It was described and termed as 'ventriculitis', 'black vomit', 'vomito negro' and 'Muskelmagenerosionen' in different published articles. GEU is characterized by erosive lesions in the koilin layer of gizzard, and macroscopic defects in mucosa (Gjevre et al. 2013). *Bacillus* species, particularly *Bacillus cereus* (*B. cereus*) group, is being widely applied as probiotics in plant pesticides, animal feed or human food chains. However, some pathogenic *B. cereus* isolates have been recently proved to be responsible for GEU in birds and have potential hazards to cause vomiting, diarrhea or ulceration in birds, pigs and human as well (Li et al., 2020; Zuo et al. 2020, Haque et al 2021a). In another study, a pathogenic isolate of *B. cereus* was obtained from a diseased hen with hemorrhaged pneumonia and its feed. Subsequently, typical diarrhea and GEU were determined after post oral administrations in chickens. Moreover, its genome contained virulence genes which mediated intestinal cell death and host immune system dysfunction (Zhang et al., 2019). When *B. cereus* along with some other pathogens co-infected the chicken and ducks, it caused GEU, and sometimes lead to respiratory infection with immunosuppression, promoted severe diarrhea and malnutrition (Zuo et al 2020, Haque et al 2021b, Haque et al 2022).

Epidemiology of GEU in Poultry Worldwide

GEU has been reported in many countries across the world. It affects various domestic birds with a prevalence of 1-50 % depending on species, region and etiological factors. The distribution of GEU is well summarized in Table 1. It is worth noting that in American and Asian countries, 20-30% commercial broilers have been identified to develop mild to moderate gizzard erosions (GE) (Contreras 2016). In China, GE is commonly found in fast growing broilers, yellow-feather broilers, and ducklings. It resulted in acute diarrhea and malabsorption within the first with an average 15% mortality (Wang et al. 2021). In Bangladesh, the prevalence of GE in broilers has reached 70.6-87.2% (Moula et al. 2020). In regions of Southeast Asia and South America, gizzard ulceration (GU) is emerging in the field again, which highlights the global threat to poultry industry (Kichou et al. 2020).

Etiology of GEU in Poultry

There are multiple factors playing a role in the development of GEU, including congenital factors, starvation, malnutrition, feed material, toxicants (copper sulphate, biogenic amines, gizzerosine and mycotoxin, such as T-2 toxin, MAS, DAS, DON) and viral/bacterial infection (Fig. 1). Microbial invasion accounts for a large proportion of GEU epidemics. In previous studies, fowl adenovirus (FAdV-1, FAdV-4, FAdV-A) has been linked to GEU in broilers and layers in Japan, South Korea, Germany, Poland, Italy, Sweden and China (Ono et al. 2001; Lim et al. 2012; Schade et al. 2013; Zhang et al. 2017; Domanska-Blicharz and Bartczak 2019; Wang et al. 2021; Lindgren et al. 2022). The opportunistic bacteria *Clostridium perfringens* was also identified in commercial hens with gizzard ulcerations (Wang et al. 2021). In another study, *B. cereus*, co-infection with avian influenza virus H9N2 or *Chlamydia psittaci* induced GEU, respiratory distress and pneumonia in SPF chickens (Zhang et al. 2019; Zuo et al. 2020). Gastric ulcers are initiated and exacerbated by increased acid secretion and a weakened mucosal barrier (Al-garni et al. 2021). Feed microbial contamination will generate the majority of biogenic amines (histamine) which catalyzes the secretion of gastric acid. Histamine is also a potential toxic chemical to livestock and poultry as well. Besides this, gizzerosine produced in poor grade fish meal is even a more effective stimulator of gastric acid secretion in poultry (Smith et al. 2000). Malnutrition status like deficiency of vitamin B6, vitamin E and decreased sulphur-containing amino acids are

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Table 1: Prevalence of GEU in different countries during last two decades

Year	Country /region	Affected animal type	Age of bird	Prevalence	Mortality	Clinical signs (CS)	Postmortem lesions	Causal agent	Reference
1998	Japan	Broiler	51 days	9-11%	2%	No apparent CS	Ulcerated gizzard	FAdV-1	Ono et al. 2001
1999	Japan	Broiler	55 days	4-50%	3%	No apparent CS	GE	FAdV-1	Ono et al. 2001
1995-2006	Italy	Broiler	42-63 days	n.a.	n.a.	No apparent CS	Variable size of GE with brown or black discoloration	FAdV-1	Manarolla et al. 2009
2007-2008	Nepal	Pullets and broiler	2-10 weeks	n.a.	1-10%	n.a.	Hemorrhagic proliferative proventriculitis and GE	*Penicillium* spp. *Candida* spp. *Salmonella* spp. *E. coli* and *Staphylococcus* spp.	Karki et al 2009
2010	Bulgaria	Broiler	38 days	n.a.	7%	n.a.	Typical necrotic pseudomembranous inflammation of the gizzard mucosa	*Clostridium perfringens*	Dinev 2010
2010	Korea	Layer	150 days	1-2%	0.2%	Dullness, anorexia and emaciation	Dilated proventriculus and gizzard with bloody fluids, GEU	FAdV-1, K181 strain	Lim et al. 2012
2011	Germany	Broiler	27-32 weeks	5-10%	2.2-9%	n.a.	Variable areas of GEU with brown or black discoloration in the koilin layer	FAdV-1	Grafl et al. 2012
2011	Germany	Broiler	15-36 days	n.a.	3.6-8%	A reduced daily growth	Detached koilin layer with multifocal ulcerations and bloody exudates	FAdV-1	Schade et al. 2013
2013	USA	Broiler	3-6 days	n.a.	20-24%	Lethargic, moribund, lying down, with greenish loose droppings	Multifocal erosions in the koilin and proventricular-ventricular junction	Tribasic Copper Chloride	Malinak et al. 2014
2013-2014	India	Layer	12 weeks	n.a.	10-30%	Dullness, uneven growth, decreased appetite	Detach koilin layer and blackish discoloration of gizzard contents, GE	FAdV-1	Bulbule et al. 2016
2012-2015	Poland	Layer	19-73 weeks	n.a.	0.05-0.4%	n.a.	Haemorrhagic changes in provetriculus and destruction of the koilin layer	FAdV-A	Domanska-Blicharz and Bartczak 2019
2015	USA	Quail	2-3 weeks	n.a.	n.a.	Loose droppings, depression	Irregular dark brown fissures within the koilin layer	FAdV-1	Kumar et al. 2021
2009-2016	Great Britain	Pullets and layer	6 weeks, 21-23 weeks	n.a.	0.12–0.3%	No apparent CS	Different of GEU with discoloration of the koilin layer	FAdV-1	Grafl et al. 2018
2016	Poland	Turkey	2 days	n.a.	7.3%	n.a.	GEU with dark green color of the koilin lining and congestion of the gizzard	Copper sulphate	Giergiel et al. 2019
2016	Sweden	Broiler	9-10 days	n.a.	n.a.	Decreased growth and uneven growth	Detached koilin layers, discoloration, bleeding, GE	FAdV-1	Lindgren et al. 2022
2016-2018	India	Layer	9-13 weeks	n.a.	0.3-7.7%	Dullness, reduced appetite and growth	Mild to severe GE with blackish discoloration of gizzard contents	FAdV-2 and 3	Chitradevi et al. 2020
2008-2019	Diverse region	Broiler and layer	23-189 days	11%	n.a	n.a	Characteristic GEU	FAdV-A	Kiss et al. 2021
2019	Iran	Broiler	16 days	5–10%	6%	Decreased growth	Severe GE with multiple black-brown areas of variable size in the koilin layer and mucosal inflammation	FAdV-1	Mirzazadeh et al. 2021

GE=Gizzard erosions, GEU=Gizzard erosions and ulcerations, n.a.=Not available

associated with gizzard ulceration as well (Dinev 2010). Furthermore, mycotoxins will exacerbate hatching chickens and survival of offspring. In recent report, FB1 or FB1+DON induced poor hatchability, an increasing mortality of chicks, GEU and hemorrhagic pneumonia (Wang et al. 2021). Nevertheless, the etiology of GEU is still not well-known, various studies of GEU are summarized in Table 2.

Pathogenesis of GEU in Poultry

Due to the complexity of GEU etiology, its pathogenesis varies from case to case. Digestive disorders in poultry mostly involve the changes of gastric secretions, either hyper- or hypo-secretion affects the internal milieu of gastrointestinal tract (Hoerr 1998). A hypothetical mechanism of GEU caused by *B. cereus* in poultry is illustrated in Fig. 2.

Table 2: Studies in the research of GEU etiology

Year	Animal model	Age of bird	Exposure period	TCID50 or CFU/ml or mg/kg or diet and route	Main findings	Pathogen/ Causal agent	Reference
2002	Broiler chicken	7 days	5 days	0.65-1.15 ppm, orally	Pervasive alterations in the gizzard and proventiculus with acute cuticle erosions and lamina propria swelling	Gizzerosine	Tisljar et al. 2002
2005	Layer chicken	28 weeks	3 weeks	2.5-10 mg/kg b.w. daily, orally	Discolorations and GEU of gizzerosine-treated bird compared to control group	Gizzerosine	Artuković et al. 2005
2006	Broiler chicken	day 1	35 days	n.a.	Bacterial number rose considerably with the severity of mucosal gizzard injuries	*Clostridium perfringens*	Novoa-Garrido et al. 2006
2010	SPF chicken	7 days	21 days	106, orally	Chicks had focal gizzard ulcers including a course or hazy koilin layer	FAdV-1	Lim et al. 2012
2013	Broiler chicken	day 1	17 days	107.8, Orally	Gross and histopathological alterations may be associated with an increased viral load in gizzards	FAdV-1	Grafl et al. 2013
2014	Layer chicken	4 weeks	21 days	107.2, IP	GEs are linked with adenovirus in commercial layer chickens	FAdV-C, D and E	Bulbule et al. 2016
2016	Pullets	21 days	14 days	107, Orally	FAdV-1 infected birds exhibited distinctive pathomorphological alterations in gizzard	FAdV-1	Grafl et al. 2018
2017	Chicken embryo	11 days	20 days	FB1=6-24 µg/egg FB1+DON=0.1+(3-12) µg/egg	FB1 and FB1+ DON contamination are linked to reduced hatchability and GUs in chicks	Mycotoxin FB1 and DON	Wang et al. 2021
2019	SPF chicken	Day old	21 days	1x108, IG	Feed-borne *B. cereus* exposure could worsen GU and promote H9N2-induced pneumonia	*Bacillus cereus*	Zhang et al. 2019
2019	Layer chicken	20 weeks	14 days	108, Orally	The major pathogens and the cause of AGE in layers are FAdV-1 strains	FAdV-1	Grafl et al. 2020
2019	Pullets	Day old	14 days	107, Orally	Infected birds Characteristic lesions and histopathological changes of AGE, as well as reduced growth	FAdV-1	Mirzazadeh et al. 2021
2020	SPF chicken	1 week	42 days	1x108, orally	*B. cereus* produced a significant GEU as well as promotes prolonged chlamydial infection and aggravates immunological reactions	*Bacillus cereus*	Zuo et al. 2020

TCID50=Tissue culture infective dose, IP= Intraperitoneal, IG=Intragastrically, n.a.=Not available, GE=Gizzard erosion, GU=Gizzard ulcer, GEU=Gizzard erosion and ulceration, AGE=Adenoviral gizzard erosion.

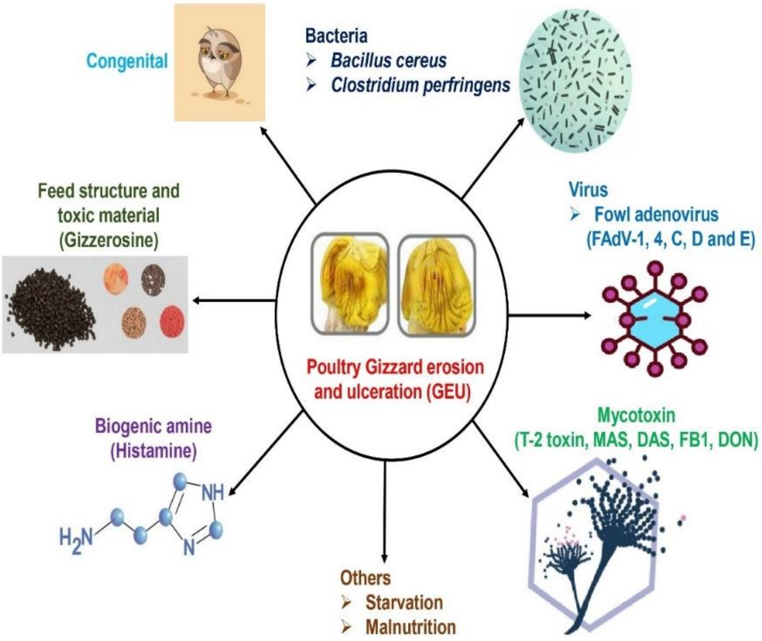
Congenital
Bacteria
➢ Bacillus cereus
➢ Clostridium perfringens
Feed structure and toxic material (Gizzerosine)
Virus
➢ Fowl adenovirus (FAdV-1, 4, C, D and E)
Poultry Gizzard erosion and ulceration (GEU)
Biogenic amine (Histamine)
NH
H_2N
N
Mycotoxin (T-2 toxin, MAS, DAS, FB1, DON)
Others
➢ Starvation
➢ Malnutrition

Fig. 1: Etiology and predisposing factors of GEU in poultry (Smith et al. 2000; Tisljar et al. 2002; Ono et al. 2003; Dinev 2010; Gjevre et al. 2013; Schade et al. 2013; Contreras 2016; Zhang et al. 2019; Li et al. 2020; Zuo et al. 2020; Haque et al. 2022)

Pathogenic *B. cereus* isolates harbor a set of virulence factors, including emetic/diarrheal toxins (Table 3) and tissue-destructive enzymes (PI-PLC, PC-PLC, SMase, β-lactamase, InhA1, NprA). However, the specific molecular mechanisms of most toxins remain unclear (Haque et al 2021a). *In vivo* data demonstrated that the emetic toxin, cereulide, interacted with serotonin 5-HT3 receptor to facilitate proapoptotic or necrotic factors, thereby inhibiting ATP formation,

Table 3: Toxins of *B. cereus* (Ehling-Schulz et al. 2006; Fagerlund et al. 2010; Haque et al. 2021a; Haque et al. 2021b)

Toxins		Properties	Toxicity	Encoded genes	Prevalence (%)
Emetic toxin	Ces	K+ ionophore, heat-stable, highly lipophilic cyclopeptide (1.2 kDa)	Hepatic and immune dysfuntion, cerebral effects, respiratory distress, bioaccumulation in vital organs and tissues, cytotoxic and mitochondriotoxic to various mammalian primary cells and cell lines, and necrotic cell death	*cesA, cesB*	1.5-32.8
Enterotoxin	HBL	Heat-labile, three component proteins, B (35 kDa), L1 (36 kDa) and L2 (45 kDa)	Intestinal fluid secretion, disrupting osmotic equilibrium, pore formation, hemolysis, cytotoxicity, dermonecrotic and vascular permeability activities	*hblA, hblB, hblC, hblD*	29–92
	NHE	Heat-labile, three component proteins NheA (39 kDa), NheB (45 kDa) and NheC (105 kDa)	Intestinal fluid secretion, transmembrane pore formation, osmotic and Vero cell lysis and cell death	*nheA, nheB, nheC*	84− 100
	CytK	Heat-labile, single-cell protein (34 kDa)	Pore formation, hemolysis, cytotoxicity (Caco 2 and Vero cells) and necrosis	*cytK1, cytk2*	37–89
	Ent-FM/ CwpFM	Single-cell protein (45 kDa)	Hemolysis, capillary permeability and cytotoxicity (Vero cells)	*entFM*	84− 100
	ENT	Single- cell protein (40/41 kDa)	Cytotoxicity, capillary permeability and diarrheal toxigenicity	*bceT*	12–75
Hemolysins	HlyI/ CLO	Heat-labile, cholesterol-binding hemolysin (52 kDa)	Pore formation, cell lysis and necrosis	*clo*	-
	HlyII	Heat-labile, β-barrel channel-forming toxin	Pore formation, cytotoxicity and apoptosis in macrophages (pathways)	*hlyII*	19–56
	HlyIII	Oligomeric pore-forming hemolysin (24 kDa)	Hemolysis and pore formation	*hly-III*	-

Ces = Cereulide; HBL = Hemolysin BL; NHE = Nonhemolytic enterotoxin; CytK = Cytotoxin K; Ent-FM = Enterotoxin FM; CwpFM = cell wall peptidases FM; ENT = Enterotoxin T; HlyI = Hemolysin I; CLO = Cereolysin O; HlyII = Hemolysin II; HlyIII = Hemolysin III.

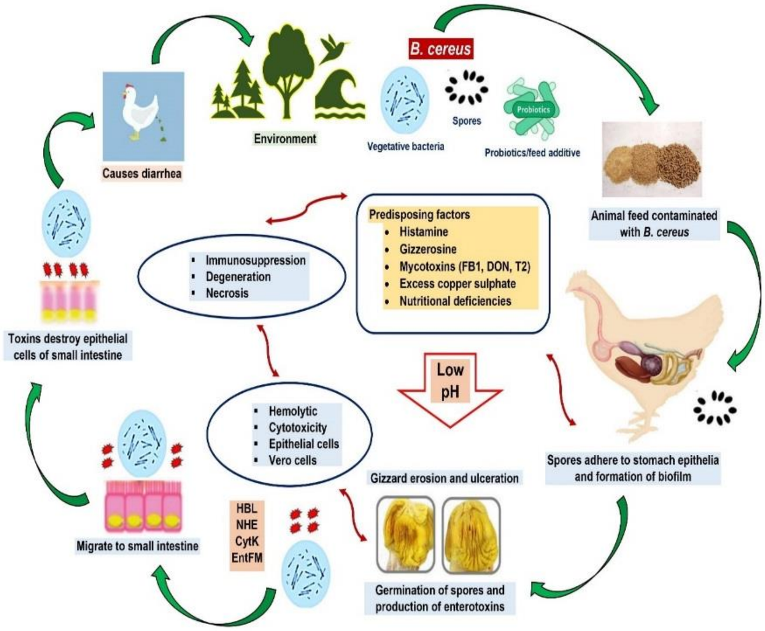

Fig. 2: Possible pathogenesis of GEU and diarrhea in poultry due to *B. cereus* infection (Zhang et al. 2019; Zuo et al. 2020; Haque et al. 2021a; Haque et al. 2021b; Haque et al. 2022)

downregulating mitochondrial function and interfering respiratory activity and lipid oxidation, leading to liver failure during its from the stomach to small intestine (Haque et al 2021a; Haque et al 2021b). *B. cereus* can tolerate a very low pH by forming spores which attach to the hydrophobic layer of gastric epithelium (Tsilia et al. 2016). The koilin layer of gizzard contains high quantities of leucine proteins and arginine which accounts for about 80% of it and may assist *B. cereus* to form biofilms, leading to stomach acid tolerance and metabolism (Majed et al. 2016; Zuo et al 2020; Haque et al 2021a). Notably, *Hbl* and *Cytk* genes are enterotoxins produced by *B. cereus* which belongs to the group of β-barrel pore-forming toxin family. These can cause dermonecrotic, cytotoxic and hemolytic effects (Ehling-Schulz et al. 2006). *B. cereus* toxins reach to the highest level during the late phase of growth and have the potential to directly destruct

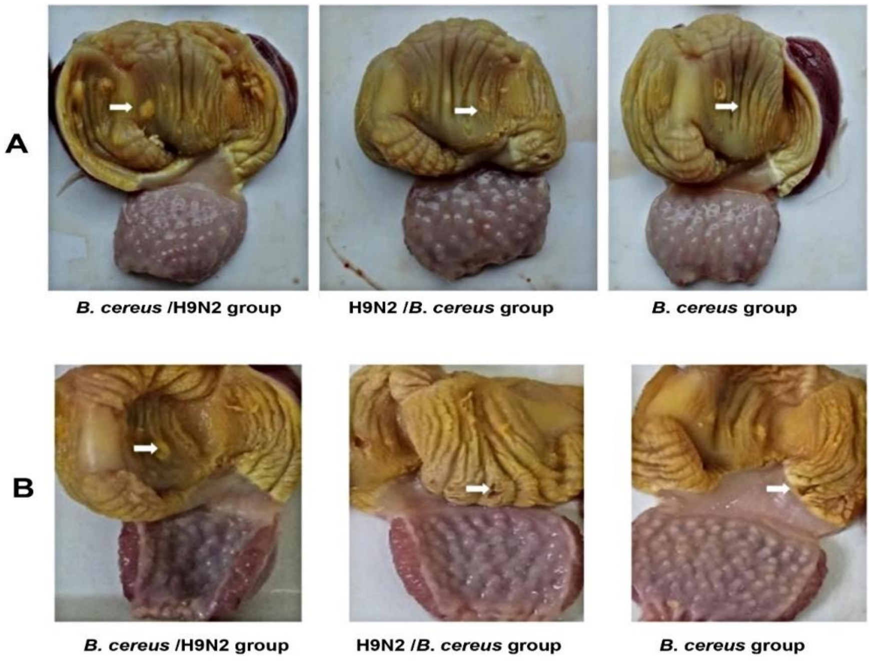

Fig. 3: The effect of *B. cereus*/H9N2 co-infection on GE by day 7 post infection. *B. cereus* or *B. cereus*/H9N2 displayed severe GEU syndrome while medium GEU was observed in the H9N2/*B. cereus* group (white arrows). B) The effect of *B. cereus*/H9N2 co-infection on GE by day 14 PI. *B. cereus*/H9N2 or H9N2/*B. cereus* displayed typical GEU and GEU recovery was observed in the *B. cereus* group (white arrows) (Zhang et al., 2019).

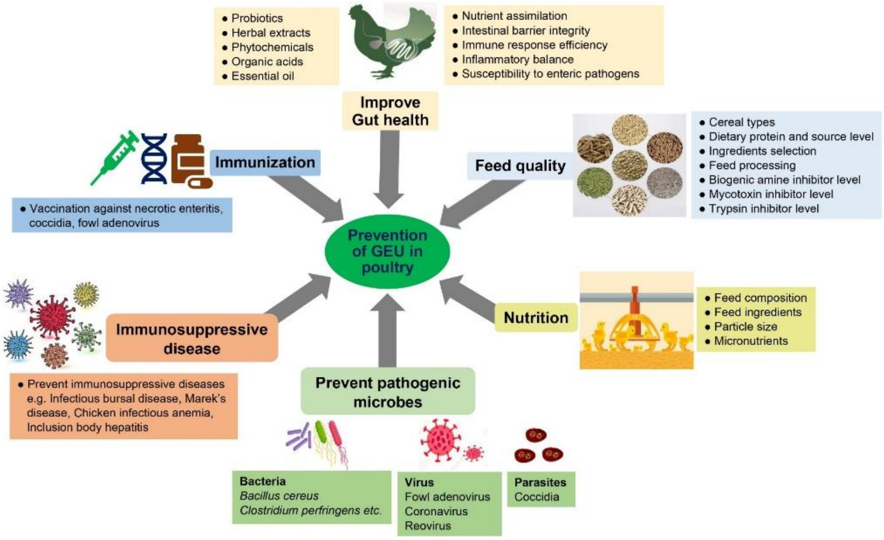
Probiotics
Herbal extracts
Phytochemicals
Organic acids
Essential oil
Nutrient assimilation
Intestinal barrier integrity
Immune response efficiency
Inflammatory balance
Susceptibility to enteric pathogens
Improve Gut health
Immunization
Vaccination against necrotic enteritis, coccidia, fowl adenovirus
Feed quality
Cereal types
Dietary protein and source level
Ingredients selection
Feed processing
Biogenic amine inhibitor level
Mycotoxin inhibitor level
Trypsin inhibitor level
Prevention of GEU in poultry
Immunosuppressive disease
Prevent immunosuppressive diseases e.g. Infectious bursal disease, Marek's disease, Chicken infectious anemia, Inclusion body hepatitis
Nutrition
Feed composition
Feed ingredients
Particle size
Micronutrients
Prevent pathogenic microbes
Bacteria
Bacillus cereus
Clostridium perfringens etc.
Virus
Fowl adenovirus
Coronavirus
Reovirus
Parasites
Coccidia

Fig. 4: Comprehensive strategies against poultry gastric erosions and ulcerations (GEU)

the koilin layer and mucosa of gizzard which alter the permeability of blood vessels and therefore resulted in gastric internal tract damage, long-term ulceration and diarrhea (Fagerlund et al. 2010; Zuo et al 2020; Haque et al 2021a). A study reported that feed-borne *B.* cereus co-infected with avian influenza virus (H9N2) or *Chlamydia psittaci* lead to serious GEU in all tested chickens (Fig. 3 A-B), which implies that the damage of the integrity of gizzard surface allows susceptible pathogens to thrive (Zhang et al. 2019; Zuo et al 2020).

Prevention and Control Strategies against GEU

Comprehensive measures are urgently needed against GEU and a series of measures are highlighted in Fig. 4. To ensure the sustaining poultry industry and food safety, microbial control is critical to GEU prevalence. Thus, antibiotic-free, alternative approaches are on highly demand for poultry industry. Probiotics, herbal medicine, plant extract and phytochemicals are documented to show some promising effects in multiple studies (Table 4).

Healthy birds with a good capability of digestion, absorption and immunity are the cornerstone to combat GEU. Therefore, good environment and proper managements are essential for GEU control. Feed industry should process animal feed in an economical and quality control ways (Smith et al. 2000). The material and size of feed needs to be carefully adjusted. Highly oat hulls in daily diet alleviates GEU effectively in broilers (Kaldhusdal et al. 2012; Moula et al. 2020), because of coarse-grained material containing non - soluble fibers,

Table 4: Probiotics/phytochemicals/herbal extract used to control gastric ulcerations

Herbal extract / probiotics / phytochemicals	Concentration in diet/Doses	Model	Group/ active ingredients	Mechanism of Gastroprotection	Percentage of GU inhibition	Reference
Stevia rebaudiana extract	Diet containing 0.2% Stevia extract (on dry matter basis)	Histamine induced GEU in broiler	Glycosides	Antioxidant activity, blocking of H2-histamine receptor, inhibition of H^+/K^+ -ATPase	-	Takahashi et al. 2001
Lactobacillus rhamnosus	10^8- 10^9 CFU /day	Acetic acid induced gastric ulcer in rats	-	Cell apoptosis to cell proliferation ratio ↓, angiogenesis ↑, ODC ↑, Bcl-2 ↑, VEGF ↑, EGF ↑	-	Lam et al. 2007
Epicatechin	25, 50 and 75 mg/kg	Ethanol/indomethacin induced GU in rats	Flavonoids	GMS ↑, H^+ secretion ↓, HSP-70 ↑, SOD ↑, NO ↑	67.8%, 57.9%, 43.8%	Rozza et al. 2012
Bifidobacterium bifidum	7.5×10^{10} CFU /mL	HCL/Ethanol induced GU in rats	-	Mucin production ↑, mucin 5ac gene expression ↑	51.1%	Gomi et al. 2013
Moringa oleifera leaf meal	Dietary inclusion of *M. oleifera* leaf meal @ 1- 25 g per kg of feed	Broiler	Polyphenols, lipids, amino acids	Antimicrobial and antioxidant activity, FCR ↑	-	Nkukwana et al. 2014
Astaxanthin (ASX)	Basal diet containing 100 ppm ASX	Histamine induced GEU in broiler	Carotenoid	Antioxidant activity, SOD ↑, MDA ↓	-	Ohh et al. 2016
Rhus tripartita stem (RSE)	200, 400 and 800 mg/kg	Ethanol induced GU in rats	Gallic acid, catechin, proanthocyanidin	pH ↑, GJV ↓, GMS ↑, MDA ↓, CAT ↑, SOD ↑, GSH ↑	82.4%, 87.5%, 95.11%	Barka et al. 2017
Lactobacillus reuteri	1×10^{10} CFU/ml	Ethanol induced GU in rats	-	GMS ↑, MPO ↓, MDA ↓, GSH ↑, NO ↑, NF-κB ↓, TNF-α ↓, IL-1β ↓, COX-2 ↓	90%	Sun et al. 2018
Pulicaria undulata subsp. *undulata* plant extract	500 mg/kg	Ethanol induced GU in rats	Polyunsaturated fatty acids, sterols, minerals, polysaccharides, terpenoids, proteins, and halogenated compounds	GJV↓, SOD ↑, GSH ↑, MDA ↓, LDH ↓, SDH ↓, AP ↓, G-6-Pase ↓, 5´NT ↓, ICAM-1 ↓, TNF-α ↓, IL-10 ↑, Protein content ↑	71.42%	Fahmi et al. 2019
Myristica fragrans seeds	200mg/kg	Ethanol induced GU in rats	Flavonoids, tannins, terpenoids, alkaloids, saponins, steroids, glycosides, diterpenes and phenols	pH ↑, GJV ↓	60.41%	Sattar et al. 2019
Juglans regia L. (Walnut)	220, 440, and 880 mg/kg	Ethanol induced GU in rats	Oligopeptides	pH ↑, GJV ↑, IL-1β ↓, IL-6 ↓, IL-10 ↑, TNF-α ↓, PGE2 ↑, NO ↑, Bcl-2 ↑, IκBα ↑, Bax ↓, Caspase-3 ↓, NF-κB p65 ↓, AST ↓, ALT ↓, MDA ↓, CAT ↑, SOD ↑, GSH ↑	-	Liu et al. 2020
Ficus thonningii aqueous extract (Fruits)	125, 250 and 500 mg/kg	HCL/Ethanol induced GU in rats	Flavonoids, tannins, gallic acids, saponins, mucilage, betacyanins	Antioxidant activity, Anti-inflammatory activity, GJV ↓, GMS ↑	70.7%, 71.8%, 95.9%	Uku et al. 2020
Euphorbia hirta crude extract + Honey	*E. hitra* @ 200, 400, and 800 mg/kg bodyweight + Honey @ 1 ml	HCL induced GU in rats	Alkaloids, triterpenoids, phytosterols, tannins, polyphenols, and flavonoids	GMS ↑, GJV ↓	94.7%, 87.9%, 87.97%	Onyeka et al. 2020
Gallic acid	10, 30 and 50 mg/kg	Ethanol induced GU in rats	Phenolic compound	pH ↑, GMS ↑, IL-1β ↓, IL-6 ↓, TNF-α ↓, PGE2 ↑, NO ↑, Bcl-2 ↑, Nrf2 ↑, HO-1 ↑, Bax ↓, Caspase-3 ↓, TBARS ↓, CAT ↑, SOD ↑, GSH ↑	17.5%, 50.2%, 57.5%	Zhou et al. 2020
Polygonum cuspidatum Root	100 and 300 mg/kg	HCL/Ethanol induced GU in rats	Anthraquinones and stilbenes	SOD ↑, GSH ↑, PGE2 ↑	82.8%, 97.9%	Kim et al. 2020a
Inula britannica L. flower	100 and 300 mg/kg	HCL/Ethanol induced GU in rats	Flavonoids and sesquiterpene lactones	GMS ↑, SOD ↑, CAT ↑, GSH ↑, MDA ↓, PGE2 ↑	94.5%, 98.7%	Kim et al. 2020b
Lactobacillus acidophilus	0.5 mg/ml (100 million CFU /ml)	Aspirin induced GU in rats	-	IL-6 ↑, IL-10 ↑, TNF-α ↓, PGE2 ↑, GSH ↑, MDA ↓, CAT ↑, SOD ↑	-	Al-garni et al. 2021
Pachira glabra L. extract	100, 1000, and 400 mg/kg	Ethanol induced GU in rats	Ferulic acid, coumaric acid, gallic acid, quercetin glycosides, maltol, methyl coumarate	NF-κB p65 ↓, COX-2 ↓, Bcl-2 ↑, Bax ↓, Caspase-3 ↓	-	El-Din et al. 2021

Brassica oleracea var. *capitata* L. extract (Cabbage)	25, 50 and 100 mg/kg	HCL/Ethanol induced GU in rats	Polyphenols, glucosinolates, vitamins and minerals	Inhibition of H^+/K^+ -ATPase, Histamine ↓, mRNA expression ↓, MDA ↓, CAT ↑, SOD ↑, NF-κB ↓, IL-1β ↓, IL-6 ↓, IL-18 ↓, TNF-α ↓, COX-2 ↓, iNOS ↓	-	Kim et al. 2021
Lactobacillus plantarum	1.0 × 10^9 CFU/kg	Alcohol induced GU in rats	-	SOD ↑, GSH ↑, MDA ↓, PGE2 ↑, SS ↑, GSH-Px ↑, TNF-α ↓, IL-1β ↓, IL-6 ↓, IL-10 ↑, Nrf2 ↑, SOD ↑, CAT ↑, GSH1 ↑	83.61%	Wu et al. 2021
Alcea kurdica extract	100mg/L	Quails	Gallic acid, quercetin, mucilage, vitamin C	HDL ↑, LDL ↓, VLDL ↓, TCHO↓	-	Jabbar 2022

ALT=Alanine aminotransferase; AP=Acid phosphatase; AST=Aspartate aminotransferase; Bcl-2=B-cell lymphoma 2; CAT=Catalase; COX-2=Cyclooxygenase-2; EGF=Epidermal growth factor; FCR=Feed conversion ratio; GEU=Gastric erosion and ulceration; GJV=Gastric juice volume; GMS=Gastric mucus secretion; GSH=Glutathione; GSH-Px=Glutathione peroxidase; GSH1= Gamma-glutamylcysteine synthetase; G-6-Pase=Gluose-6-phosphatase; GU=Gastic ulcer; HDL=High density lipoprotein, HO-1=Heme oxygenase-1; HSP-70=Heat-shock protein 70; ICAM-1=Intracellular adhesion molecule-1; IκBα=Inhibitor kappa Bα; IL-1/6/18=Interleukin-1/6/18; iNOS=Inducible nitric oxide synthase; LDH=Lactate dehydrogenase; LDL=Low density lipoprotein, MDA=Malondialdehyde; MPO=Myeloperoxidase; NF-κB p65 =Nuclear factor kappa B p65; NO=Nitric oxide; Nrf2=Nuclear factor erythroid 2–related factor 2; 5´NT=5´-Nucleotidase; ODC=Ornithine decarboxylase; PGE2=Prostaglandin E2; SDH=Succinate dehydrogenase; SOD=Superoxide dismutase; SS=Somatostatin; TBARS=Thiobarbituric acid reactive substances; TCHO=Total cholesterol; TNF-α=tumor necrosis factor alpha; VLDL=Very low density lipoprotein, VEGF=Vascular endothelial growth factor

which helps the digestion of gizzard. Moreover, hygiene surveillance should be included to check microbial contaminations in feed and raw stuff and human food chains transmitted from animal feed. Once toxic biogenic amines (histamine, gizzerosine) residual in the feed, these are unable to remove by heating approaches. Although astaxanthin, a natural antioxidant, has potential to improve gastric-intestinal tract during GEU and stress (Ohh et al. 2016), it also keeps feed and raw material in hygienic condition during the period of processing or storage (Feddern et al. 2019).

Probiotics compete with pathogens for niches in gastric internal tracts, thus these are able to interfere infections and alter host's metabolism. *B. cereus* has widely contaminated in animal diets, however, the limited knowledge and improper isolation and identification of different strains, may let virulent toxins be overlooked and promote the potential transfer of antibiotic resistant genes among microbes. Therefore, virulence analysis on gene level and generally recorded as safe (GRAS) regulation should be combined together to decide whether *B. cereus* probiotic can be used as feed additive or being eliminated from animal diets (Haque et al 2021b). Moreover, feed fermentations are abused for growth promotion using too many probiotics. The process without inspection and supervision may facilitate *B. cereus* to synthesize toxins. For this reason, protocols for processing and fermentation of raw material need to be standardized and assessed by animal nutritionists and veterinary experts. Moreover, some material with antimicrobial activities such as asteraceae, roselle, rosemary, clove and thyme can be used to control pathogenic *B. cereus* in poultry (Haque et al. 2021a). Acidifiers (organic acids and their derivate) have been utilized to regulate gut microflora, enhance immunity, improve feed conversion ratio (FCR) and body weight. Synergistic organic acid and compounds like phytochemicals or permeabilizers are regarded as the novel measures to control infections (Pearlin et al. 2020; Haque et al. 2021b).

Herbal medicines are regarded as for GEU and resources of new drugs against digestive disorders. Herbal medicines are widely practiced, accessible, effective and safe in routine administration (Onyeka et al. 2020). Many studies have demonstrated that herbal plants, such as *Carica papaya*, *Zingiber officinale* Roscoe, *Musa paradisiaca*, *Allium sativum*, *Moringa oleifera*, *Rhus tripartita Pulicaria undulata* subsp. *undulata*, *Myristica fragrans*, *Brassica oleracea* var. *capitata*, *Pachira glabra*, *Polygonum cuspidatum*, *Inula britannica*, *Juglans regia*, *Stevia rebaudiana* and *Alcea kurdica* are capable of reducing ulcerations and gastric syndrome (Takahashi et al. 2001; Nkukwana et al. 2014; Barka et al. 2017; Fahmi et al. 2019; Kayode et al. 2019; Sattar et al. 2019; Liu et al. 2020; Kim et al. 2020a; Kim et al. 2020b; El-Din et al. 2021; Kim et al. 2021; Jabbar 2022). Feed-borne mycotoxins (FB1, DON, AFB1, OTA) negatively impact on the integrity of intestinal epithelium and cause necrotic enteritis as well as GEU in broiler chicks. A study reported severely impaired feed intake and growth of chickens and decrease in feed consumption and average body weight gain up to 12% and 14%, respectively during GEU development (Wang et al 2021). In a recent report, protocatechuic acid (PCA), a naturally occurring phenolic compound, increased the hatchability and decreased GEU of chicks caused by FB1-contaminated corn and feed (Wang et al. 2022). Furthermore, PCA exhibited significant antimicrobials against gram-positive ~~and~~ gram-negative bacteria, and fungi. It also improves humoral and cellular immune responses, suggesting a prospective usage in poultry industry (Guo et al 2018; Wang et al 2022).

Conclusion

The epidemic GEU in poultry across the world indicates the transmissible factors playing a key role in feed or food chain.

Considering the immense threat posed by GEU, the overwhelming majority of contaminations are microbial and toxin contaminations in animal feed. GEU impairs the digestion and immune responses in animal industry, and also exacerbates secondary infections and poisonings, which endangers food safety and public health. In order to prevent and control GEU in poultry, long-term monitoring and surveillance are necessary along with adopting a set of measures.

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Lactose Intolerance and Emerging Dairy Allergies as Public Health Perspective

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INTRODUCTION

Milk is a complete diet having a biggest source of lactose that provides energy to the consumers. Lactose is digested by the enzyme named as lactase (Vandenplas 2015). Some people are deficient with the normal level of lactase that leads to the lactose malabsorption. These individuals simply suffer with lactose intolerance (Martínez Vázquez et al. 2020). Severity of lactose intolerance depends on the amount of lactase present in the intestinal flora and gastrointestinal tract for absorption of lactose (Catanzaro et al. 2021). Globally, 57% of the people suffer from lactose intolerance just because of lacking lactase enzyme (Catanzaro et al. 2021). Keeping in view the prevalence record of lactose intolerance, Africa is on the top with 100% rate of affected people (Lember 2012). In Asia 70% people are prone to the lactose intolerance and dairy allergies from the milk (Lember 2012). Considering the data from American region it was just 15% among the whites and the rate was higher in African-Americans as 80% (Lember 2012; Storhaug et al. 2017).

Milk and dairy products always remain valuable sources of nutrients for young as well as adult mammals (Corgneau et al. 2017). Milk contains carbohydrates (carbohydrates and other oligosaccharides) in addition to proteins, fats, vitamins and minerals to enhance the growth of probiotic bacteria in children's intestine, mainly *bifidobacteria,* protecting infant's gut from infections. Milk lacks only a few vitamins and iron, so, it is also a perfect food for adults (Franzè and Bertelè 2010). But, in adult age, everyone cannot digest this food, resulting in intolerance (Matthews et al. 2005; Harrington and Mayberry 2008). Lactose intolerance results in pain, distended abdomen, diarrhea and flatulence (because of mal-absorbed lactose), that needs to be differentiated from other subclinical conditions, like lactose maldigestion and lactose malabsorption (Matthews et al. 2005). Lactose is only present in milk and milk derived products of mammals. It is formed by lactose synthetase system in mammary glands, that binds D-galactose to D-glucose by β-1,4 glycosidic bond. Lactase (a β-galactosidase) is required for digestion of lactose. It is found on the upper surface of microvilli's in small intestine, and more expressed in mid jejunum, where less fermentation occurs due to low bacterial population. It hydrolyses lactose to glucose and galactose, that upon digestion, get absorbed quickly by intestine and utilized as energy source and as a component of glycolipids and glycoproteins, respectively (Franzè and Bertelè 2010).

After weaning, lactase expression is down regulated in the enteric cells. It results in Lactase Non-Persistence (LNP), which bears least severity and is encountered the most (Wilt et al. 2010). When lactase deficiency is faced, lactose maldigestion occurs leading to lactose malabsorption, and it refers to a clinical condition exhibiting gastro-intestinal symptoms (Matthews et al. 2005; Misselwitz et al. 2013). The symptoms may be gastro-intestinal or extra-intestinal (Matthews et al. 2005). Lactose Malabsorption almost always result from lactase deficiency (Flatz 1987; Wilt et al. 2010). The symptoms that are consistently seen in lactose-intolerant individuals include diarrhea, bloat, nausea and abdominal pain (Deng et al. 2015). The amount of mal-absorbed lactose decides the clinical picture of lactose

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malabsorption. The clinical symptoms are ignored by a number of patients suffering from Lactose malabsorption and Lactose non-persistence if small quantity of lactose is taken (Wilt et al. 2010). The lactase expression varies over time in life; it is present throughout the intestinal mucous membranes during 8th week of pregnancy; increases up to 34th week, and at birth, it goes to its highest concentration (Deng et al. 2015). Few months after birth, enzyme activity starts to reduce, until it ends up (Franzè and Bertelè 2010). Considerable amount of lactase is synthesized during first year of life. Later, lactase synthesis is genetically reduced in LNP patients. Lactose-intolerance (LI) begins somewhere between 2 to 3 years and completes by 5 to 10 years of age, with exceptions reported in some cases(Swallow 2003). A significant part of human population possesses enzyme functionality throughout the life, but other mammals show unusual lactase maintenance (Deng et al. 2015). The incidence of LNP is greatly associated with ethnicity (Welsh et al. 1978). Generally, 70% to 100% of maldigesters originate mostly from conventional non-milk consuming regions. Whereas those from milk consuming regions possess less prevalence of LM (Caballero 2005). Functionally, it should be highlighted that 50% of normal enzyme activity is sufficient for lactose digestion (Franzè and Bertelè 2010).

Abdominal distension caused by lactose fermentation by the microflora in the gut and the osmotic effect that occurs due to the presence of lactose molecules in GIT are the pathogenic mechanisms resulting in above mentioned symptoms. In a few of LI patients, it has also been reported that some strains of micro-organisms reduce carbon-dioxide to methane that can lead to constipation (Misselwitz et al. 2013). Headache, asthenia, joint/muscle pain, skin lesions, loss of concentration and mouth ulcers are the most common extra-intestinal symptoms that have been reported in many cases (Matthews et al. 2005). 2.6 L carbon-dioxide and 4 L of hydrogen gas are released by the degradation of 12.5 g of lactose (Wolin 1981). Whereas, the normal amount of gas excreted is lower than 1 L of gas per day (Tomlin et al. 1991). Yet, the presence of systemic syndrome by lactose-intolerance is in debate, and the mechanism of pathogenesis has also not been distinctly explained. As the systemic and gastro-intestinal symptoms appear many hours post consumption of lactose containing food and there are a wide variety of even non-dairy products containing lactose, which are used as additives in food, the patient is not always believed to develop these symptoms after consuming lactose (Dainese et al. 2014). There are various factors that decide the threshold level of lactose in different individuals that includes amount of lactose ingested, lactase activity level, food matrix (taken with other diet components), time of stay in gut, and intestinal micro-floral composition (Deng et al. 2015).

Although, ingestion of lactose does not enhance the expression of lactase, it has been reported that tolerance may get improved by regularly taking small quantities of lactose. This way, the intestinal microflora become adapted to lactose (Shaukat et al. 2010). So, administering the increasing amounts of lactose can regularly address the symptoms in patients of lactose intolerance (Hertzler and Savaiano 1996; Briet et al. 1997). Modulating the enteric micro-environment by enhancing the colonization of β-galactosidase activity harboring strains, could be an efficient treatment strategy for lactose intolerant subjects (Fassio et al. 2018). The symptoms relieving strategies include intake of lactose-free food components or the products having surplus amounts of probiotic bacteria, as well as the usage of exogenic enzyme formulations (Corgneau et al. 2017). The difference of lactose digestion in normal individual and lactose intolerant individual has been shown in Fig. 1.

Calcium Intake and Diet Competence

It is a well-known fact that calcium is enormously beneficial and a consolidative element of body, the skeleton alone comprising 99% of body's calcium (Emkey and Emkey 2012). The calcium homeostasis in plasma is necessary for sustaining activities of life, like maintaining skeleton, hormonal regulation, nerve impulse conduction and vascular functions (Del Valle et al. 2011; Brini et al. 2013). Calcium in diet gets absorbed in small intestine, and most of it accumulates in bones via circulation. The unabsorbed calcium excretes out of body in feces and urine (Del Valle et al. 2011).

Dairy has been a significant mean of calcium in diet and hence studied widely. 120 mg/ 100 ml Ca is present in bovine milk. In North America and Europe, dairy products account 75% of dietary calcium, vegetables and fruits 15%, mineral water 5% and other foods account rest of amount (Guéguen and Pointillart 2000; Fishbein 2004). Under normal conditions, 40% of Ca is absorbed from dairy products, so the bioavailable is the amount which is absorbed by small intestine and help in mineralizing bones (Melse-Boonstra 2020). Physiological factors (growth, pregnancy and lactation related) determine Ca storage in bones, and several hormones (PTH, calcitonin, calcitriol and estrogens) regulate it.

Calcium is mainly absorbed by intestine via passive diffusion, whereas at low and modest calcium intake, active transport occurs under Vit-D regulation (Guéguen and Pointillart 2000). Vit-D fortified milk has been shown to improve Ca absorption (Kaushik et al. 2014). Many of the components in dairy sources help in Ca and other substances' absorption by passive diffusion such as casein, phospho-peptides, lactose, phosphorous and whey proteins. Many dairy components inhibit the Ca uptake as well (Table 1). Lactose acts as a prebiotic, as it increases the production of *bifido-bacteria* that trigger Ca absorption in cecum and colon and maintain low pH (Shi et al. 2001; Szilagyi 2002; Whisner et al. 2013). Ca absorption from vegetables depends upon the phytate and oxalate content (being higher in spinach, cabbage, beetroot, broccoli, sprouts) in these. These bind with cations ($Ca^{2+,}$ Mg^{2+}, Zn^{2+}, and Fe^{2+}) and form indigestible and insoluble complexes, restricting their

Table 1: Different Components of dairy food sources affecting Ca Absorption

Sr. No.	Food Element	Effect on Ca Bioavailability	Reference
1	Vit-D	Enhances active Ca absorption at its low and modest level	(Guéguen and Pointillart 2000)
2	Phospho-peptides and Casein	Enhance passive diffusion, they bind with Ca resulting in its slower release into solution in chyme	(Mykkänen and Wasserman 1980, Lee et al. 1983; Li et al. 1989)
3	Proteins (α-lactalbumins and β-lactoglobulins)	Same effect as Phospho-peptides and Casein	(Guéguen and Pointillart 2000)
4	Amino Acids (L-Arginine and L-lysine)	Same effect as Phospho-peptides and Casein	(Lee et al. 1983)
5	Lactose	Enhances passive diffusion due to broadened paracellular spaces in intestinal cell lining*	(Bronner 1992)
6	Phosphorous	Decreases absorption-it binds Ca to undigestible food complexes. In distal part of nephron, re-absorption increases. Increases uptake of absorbed Ca into bones.	(Guéguen and Pointillart 2000)
7	Sulphonated Proteins	Negative Ca balance due to hypercalciuria induction	(Guéguen and Pointillart 2000)
8	Fat	No effect on Ca absorption. It forms insoluble soaps with Ca that dissociate at acidic gastric pH	(Allen 1982)

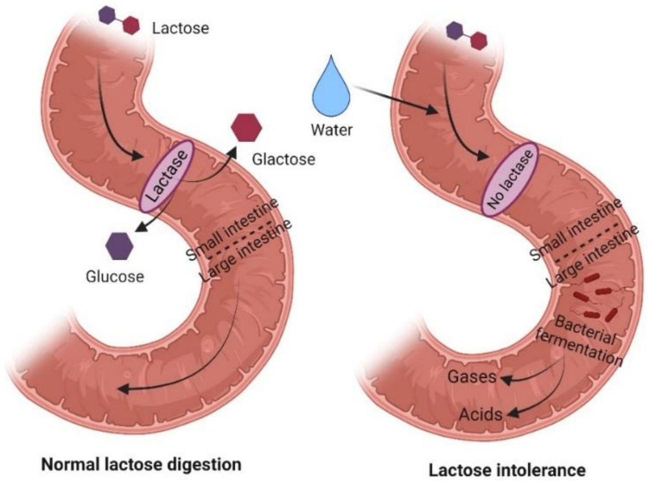

Fig. 1: Difference of Normal Lactose Digestion and lactose Intolerance in Intestine (Created on Bio render)–Created by Biorender

bioavailability (Melse-Boonstra 2020). Adults face negative Ca balance after they achieve peak bone mass (~35y) and ~10 mg Ca is lost every-day. In post-menopausal women, this loss may reach 40 mg /day or more (Avioli 1984; Arasi et al. 2022).

According to studies, it happens at higher lactose concentrations.(Pansu et al. 1981; Cochet et al. 1983; Krebs et al. 2015), the amounts present in milk and dairy products are less likely to promote much absorption (Tremaine et al. 1986; Griessen et al. 1989).

Lactose Malabsorption

Milk acquires a great importance in the human diet and is an essential part of diet since thousands of years due to its high nutritive value and the variety of nutrients incorporated in it (Harrington and Mayberry 2008). One of the important nutrients in milk is lactose which is commonly called as "milk sugar" and is found in various dairy products. Except for walruses and sea lions, lactose is the major source of carbohydrate in milk of majority of the mammals including humans (Reich and Arnould 2007). The significance of lactose lies in the fact that it is a major precursor of many important biological metabolites which are vital for the human life. For instance, lactose is essential for the formation of glucocerebrosides; the molecules which are considered the vital components of nerve cells being the part of plasma membranes of nerve cells (Luna and Gallardo 2007). Lactose (β-galactosyl-1,4 glucose) is basically a disaccharide which is further made of galactose and glucose found in milk and is absorbed and digested in the small intestines of humans. Prior to its absorption, lactose needs to be hydrolyzed in the gastrointestinal tract of humans into two components with the action of an enzyme known as lactase. The enzyme lactase-phlorizin hydrolase (lactase) is mainly expressed in the brush border of the intestinal villi where it resides on the membrane of enterocytes (Misselwitz et al. 2019; Usai-Satta et al. 2022). Two identical polypeptide chains of size 160 kDa are combined with the intra cytoplasmic part to make up this enzyme and it shows the optimum activity at a pH between 6 and 8 in the small intestine. Lactose malabsorption occurs when the activity of lactase is reduced or declined which may be the result of deficiency of this enzyme in the intestinal cells; commonly referred as lactase deficiency. Any damage to the mucosal wall of intestine may cause lactase deficiency or even lead to failure of this enzyme to express and hydrolyze the lactose into absorbable components (Misselwitz et al. 2019). In a study, the results showed that Japan, Vietnam, and Uganda had the highest prevalence of lactose malabsorption (100%), while it was the lowest (14%) in the Central Italy (Ingram et al. 2009; Swallow 2003).

The metabolism of lactose is highly dependent on the expression and activity of lactase enzyme. Malabsorption of lactose is mainly subjected to the lactase deficiency which can be further classified in to 3 categories viz, congenital, primary and secondary (Bouchoucha et al. 2021). The other name used for lactase deficiency is hypolactasia. The most frequently occurring condition is primary hypolactasia in which the the activity of lactase at the brush border of intestine decreases progressively and is wholly due to the autosomal recessive condition. C/T-13910 is a single nucleotide polymorphism that may have important role in developing lactose malabsorption in specific populations around the world (Schirru et al. 2007). Another type of hypolactasia is the congenital which occurs due to an autosomal recessive condition and is very rare to be found in most of the populations (Anguita-Ruiz et al. 2020).

Lactose Intolerance

There is a common misunderstanding among the people regarding the term's lactose malabsorption and lactose intolerance and in fact, the latter is not synonymous with lactose malabsorption. Lactose intolerance is a manifestation of symptoms which occur due to the malabsorption of lactose from the small intestine. Lactose intolerance is a condition that is characterized by the occurrence of abdominal signs including diarrhea, abdominal pain, bloating, and borborygmi (Zhao et al. 2010). It must be noted that the development of abdominal signs is not compulsory to every patient of lactose malabsorption because many patients show no signs of intolerance after ingesting lactose in their diets. However, the presence of lactose malabsorption is a pre-requisite or necessary condition in order to initiate the onset of intolerance or abdominal signs. The severity of lactose intolerance or more specifically the occurrence of abdominal signs is dependent on various intrinsic and extrinsic factors including the amount of lactose intake in the diet, the intake of other food items along with lactose diet which can influence the bowl transit time, the rate at which the undigested lactose is transferred to large intestine, and most significantly the expression and activity of lactase enzyme in the brush border of the small intestine (He et al. 2008; Windey et al. 2015). Regarding the threshold levels of lactose for causing lactose intolerance in patients, a study revealed that half pint or 8 oz of milk was enough to cause cramps or develop bloating in almost 54% participants in the study (Bayless et al. 2017; Mitchell et al. 1975). Several diagnostic tests are available for the detection of the lactose malabsorption and lactose intolerance in different populations.

Lactase Non-Persistence in Humans

Except humans, all other adult mammals show considerable decline in the production of intestinal lactase and is considered to occur just after the weaning (Romero-Velarde et al. 2019). However, in some individuals, the levels of lactase remain high even after the weaning is complete leading to condition known as lactase persistence. In contrast to the previous condition, lactase non-persistence (LNP) is characterized by the comparatively low levels of intestinal lactase or decreased expression and activity of this enzyme known as hypolactasia. In case of humas, the most prevalent phenotype is LNP with a prevalence rate of 65-70% in most of the populations (Ingram et al. 2009). In LNP, there is downregulation of the lactase gene; MCM6 which is situated on chromosome 2 having a size of 50 kb. A study showed the impact of C/T-13910 and G/A-22018 variants of DNA in downregulating the expression of intestinal lactase (Enattah et al. 2002). The homozygotes are T/T 13910 and C/C 13910 responsible for lactose persistence and LNP respectively, while the heterozygote responsible for reducing the activity of lactase to half is C/T 13910 (Bayless et al. 2017). The genetic variance and the downregulation of MCM6 gene is shown in Table 2.

The prevalence of lactase non-persistence has been calculated in various studies involving various ethnic groups in order to find out the epidemiological variations in the occurrence of LNP. According to studies, the highest prevalence of LNP was found in China (100%), while in Iraqi Jews it was 93%, and was lowest in the Swedish population which was merely 10% (Bayless et al. 2017; Corgneau et al. 2017; Raz et al. 2013).

Common Cow Milk Allergies

Cow milk (CM) is nutritious biological fluid introduced into infant's diet and cow milk allergy (CMA) is most common food allergy. CMA is immune mediated response to cow's milk protein that occurs consistently with ingestion. CMA is a complex food allergy and often misconception disorder among the general public health due to confession between CMA and milk intolerance. Both of these terms for distinct disorders and require separate technique for diagnosis and strategies for management and cure. In CMA, the immune system is involved in adverse reaction to harmless milk proteins. Allergy symptoms damage tissues due to immune system aberrant inflammatory response. CMA is not a single disease because it involves a spectrum of immunological mechanisms generally classified into IgE mediated and non-IgE mediated allergy. CMA incidence varies with age. IgE mediated CMA is most frequently observed food allergen and more dominant food allergy in children's (2-6%) and decrease into adulthood (0.1-0.5%) (Ross et al. 2005; Sicherer and Sampson 2018; Arasi et al. 2022). After consuming dairy milk or items containing dairy milk, the symptoms of IgE-mediated CMA frequently start to manifest within few minutes. These symptoms may include vomiting, diarrhea, skin rash, breathing difficulty, and anaphylaxis, which can be fatal (Walsh et al. 2016). The reported cases of patients show the 5-90% skin manifestation, 60% gastrointestinal symptoms and 0.8-9% anaphylaxis (Connor et al. 2022). Various diagnostic techniques are employed for identification but available diagnostic methods have constraints that impede our capacity to understand the underlying epidemiology (Sicherer and Sampson 2018). The best technique for identification is double blind placebo controlled oral food

Table 2: The genetic variance and the downregulation of MCM6 gene

Genetic variance (Polymorphism)	Allelic Variant	Lactase Activity	Prevalence
Lactase Non-persistent (LNP)	C/C 13910	Inactive	65-70%
Heterozygotes	C/T 13910	Activity is partial	Variable
Lactose persistent	T/T 13910	10 times more activity than hypolactasia	Almost 30%

challenge (DBPCFC). Apart from this unblinded oral food challenge (OFC) is less efficient and not always appropriate in clinical practices but well validated particularly among young infants (Bøgh and Larsen 2021). The serum specific IgE measurement and skin prick tests (SPT) predict the response possibility but in isolation it’s not sufficient. The atopy patch test is also most often used for the diagnosis of atopic dermatitis that measures the milk allergen induced T cell activation. The Patients approximately 70% with IgE mediated CMA would react to whole milk products but patients can tolerate the treated products (alters in milk proteins) (Leonard 2015; Flom and Sichere 2019). There is currently no cure for CMA. Finding new therapeutic techniques in a clinical trial environment is difficult. Obstacles to the diagnosis and effective care of CMA in clinical practice include delayed diagnosis and inadequate understanding of the underlying pathophysiology (Gore and Custovic 2004). The only effective management strategy is to avoid cow's milk throughout the course of the disease. CMA patients often have allergies to various foods. Because an infant’s immune system is not fully developed at birth, and the gastrointestinal tract is still immature, the intestinal lining leakage may occur if large amounts of intact cow's milk are fed. Intervention techniques in CMA aim at three levels: 1) primary prevention (breast feeding, partially hydrolyzed formulas, probiotics) of early sensitization; 2) secondary prevention (making cow milk less allergic) of triggering allergic reactions, and 3) induction of tolerance in already sensitized patients (Pessler and Nejat 2004; Restani et al. 2002; Lund and Ahmad 2021). The use of immune modulatory food adjuvants such as probiotics is a promising new method for primary prevention. The only effective therapeutic method for CMA sufferers is to eliminate diet milk proteins. It will remain to be a focus of fundamental food allergy investigation in the future years (Ross et al. 2005; Pessler and Nejat 2004). The composition of cow milk containing major proteins and allergens has been enlisted in Table 3.

Cow milk allergy (CMA) represents first from of allergy that can induce severe and life threating reaction. There is need of safe diagnostic techniques, best therapeutic treatment and preventions. The various forms of adverse reaction to dairy milk have been shown in Fig. 2.

Benefits of Calcium and Dairy Products

Calcium is the most plentiful mineral present in human body. It is fundamental not only for bone health, but also contributes to heartbeat, muscle contractions and other metabolic activities (Jaiswal 2001). Several nutrients are needed for development of bones in early years and their preservation to lessen the occurrence of osteoporosis and fracture in adult age (Rizzoli 2014). It has been deduced that protein and the minerals (Ca, P, Mg, Mn, Zn, Vit-D and Vit-K) are vital for the maintenance of strong bones (European Commission regulation 2012). Milk and dairy products contain considerable amounts of all these nutrients except Vit-D (Thorning et al. 2016). These minerals affect bone physiology and structure. For example, protein and calcium help in mineralization of bone by forming collagen and hydroxyapatite crystals, respectively (Heaney 2009).

The current data propose that the consumption of milk and dairy products is linked to lower probability of obesity in children, and in adults. It is associated with better body composition and weight loss during restricted energy provision. Additionally, milk and dairy products’ consumption also reduces risk of type-II Diabetes Mellitus and cardiovascular diseases, such as stroke (Thorning et al. 2016). The relationship among consumption of milk in infancy and boosted growth and mineralization of bones has been postulated extensively since 1920’s (Mann 1926). Consumption of dairy products enhance the release of type-I Insulin-like growth factor, that helps in developing skeletal muscles (Caroli et al. 2011). The lactose, casein phospho-peptides or Vit-D in dairy products help in enhanced calcium absorption from products and explain their positive effects on bones (Camara-Martos and Amaro-López 2002).

A high intake of calcium and Vit-D through dairy products can lower the circulating Parathyroid Hormone (PTH), improve bone mass, and can lower bone turnover (Heaney 2009). When blood calcium level falls, PTH concentration increases, that causes bones to release calcium, that results in bone resorption and ultimately reduced Bone Mineral Density (BMD)(Renner et al. 1998). There are some likely mechanisms through which dairy products can have positive impacts considering composition and size of body. Firstly, interaction of Ca and Vit-D may affect lipogenesis and lipolysis of adipocytes and fat oxidation(Shi et al. 2001; Zemel 2002; Zemel 2003). Secondly, Ca may help in lowering absorption and enhancing excretion of fat (Christensen et al. 2009). Thirdly, Ca may help in regulating hunger and intake of food-fat (Tordoff 2001). Fourth mechanism may be associated with the positive impacts of nutritious elements in dairy products like branched chain amino acids (BCCAAs) (Zemel 2005) and medium-chain triglycerides (Marten et al. 2006; Kouvelioti et al. 2017). In developed countries, the recommended amount of dairy products by children under the age of 9 y is ≈ 500 ml and for adults is >600ml dairy/day (Dror and Allen 2014).

Table 3: Cow milk compositions of major proteins and allergens characteristics (Restani et al. 2002; Ross et al. 2005; Bøgh and Larsen 2021)

Protein	Major Protein Composition (mg/ml)	Allergen	Size (kDa)	Disulphide (S-S) bridge in immunoglobulin's
α_s1-casein	11.6	Bos d 9 (Major)	23.6	0
α_s2-casein	3	Bos d 10 (Major)	25.2	0
β-casein	9.6	Bos d 11 (Major)	24	0
k-casein	3.6	Bos d 12 (Major)	19	1
α-lactalbumin	1.2	Bos d 4 (Major)	14.2	4
β-lactoglobulin	3	Bos d 5 (Major)	18.3	3
Serum albumin	0.4	Bos d 6 (Minor)	66.3	18
Immunoglobulins	0.6	Bos d 7 (Minor)	160	No. varies according to class and subclass
Lactoferrin	0.3	--- (Minor)	80	16

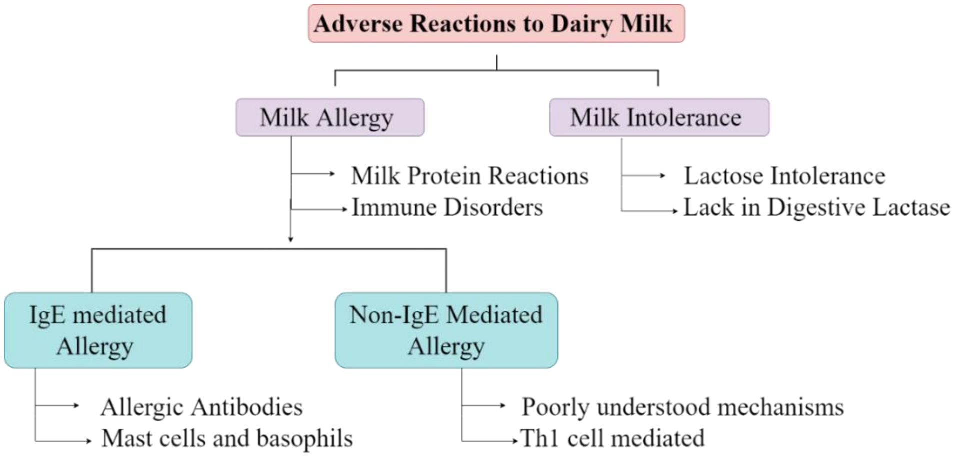

Fig. 2: Possible adverse reactions to dairy milk

Calcium in vascular smooth muscle cells regulates the blood pressure via vasoconstriction and varying vascular volume (Fardella and Rodriguez-Portales 1995; Yim and Yoo 2008). It has been reported by a systematic review of calcium supplementation and lipid metabolism that supplementing calcium reduces the low-density lipid (LDL) cholesterol and increases high-density lipids (HDL) cholesterol by suppressing the calciotropic hormones that lower the intracellular calcium in fat cells, initiating lipogenesis and fat storage (Chen et al. 2017). Furthermore, calcium in diet can also reduce the serum cholesterol level by preventing the absorption of saturated fatty acids and cholesterol (Vinarova et al. 2016). It has been found by randomized control trials (RCTs) that calcium dosage of 1200-2000 mg/day with 36-60 month treatment period can lessen the risk of recurring colo-rectal cancer (Bonovas et al. 2016). Moreover, calcium binds with bile acids in the intestinal lumen and prevents the multiplicative and carcinogenic effects of bile acids (Fardella and Rodriguez-Portales 1995; Newmark et al. 1984; Cormick and Belizán 2019).

Conclusion

Lactose intolerance (LI) and cow milk allergy (CMA) are two different terms that often used interchangeably. We should promote the appropriate use of CMA and LI terms. The prevalence of CMA is low in adults and high in infants (childhood disease). CMA is an immunological mediated reaction that affects the various body systems and can cause high mortality. LI is non-immunological reaction that's being high in dark skinned population with limited symptoms of gastrointestinal tract. LI and CMA incorporate more complex meaning than the past and their confusion leads to delay in diagnosis of CMA. LI can be managed by reducing the intake of lactose quantity but CMA can only be managed by avoiding the cow milk as strictly as possible.

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Dietary Toxicity of Heavy Metals; One Health Perspective

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INTRODUCTION

The term "heavy metals" (HMs) refers to a class of metallic elements with specific gravity larger than 4.0 and density greater than 5g/cm3 (Rehman et al. 2021) and an atomic number greater than 20 (El-Kady and Abdel 2018). Naturally, heavy metals are present everywhere but their concentration is different in many areas. Severe harmful effects occur due to the excessive use of heavy metals by living organisms (Rehman et al. 2019).

Heavy metal toxicity is a major concern fornutritional, ecological and evolutionary related reasons. The risk of heavy metals has been tremendously increased in the environment especially in agriculture setups because heavy metals remain in ground water and soil, which at certain levels leads to cause toxicity as heavy metals accumulate (Alengebawy et al. 2021; Balali-Mood et al. 2021).

Due to their widespread presence in the earth's crust, air, water, and food, heavy metal pollution poses a hazard to the ecosystem on a global scale. From coal burning facilities, smelters, and other industrial facilities, heavy metals emit airborne particles that penetrate the environment (Ali et al. 2019). Numerous human and natural activities have led to an increase in the amount of heavy metal contamination in the ecosystem. Waste disposal, unintended spills during processes, the application of agricultural pesticides and related chemicals, the transfer of contaminants into productive land through the inhalation of fumes, the movement of soil, the inhalation of dust, or the dispersion of sewage mire are just a few examples of these activities. The environment is contaminated by all of these sources (Alengebawyet al. 2021). The major source of pollution of heavy metals (HMs) in vegetables is the presence of heavy metals such as lead, nickel, cadmium, zinc, and mercury in pesticides (Zhong et al. 2018). Most of the metals e.g. copper, manganese, selenium and molybdenum have beneficial effects on human health (Ali et al. 2019).

On the basis of the importance of heavy metals in biological systems, they are classified into two categories such as essential and non-essential. Essential heavy metals are those which play an important role in biological metabolism processes while no3n-essential heavy metals don't have biological functions in living things and are non-essential to metabolic processes (Edelstein and Ben-Hur 2018; Rai et al. 2019). The toxicity of essential heavy metals depends on the concentration of exposure (Aliasgharpour2020) and includes the most important elements which are manganese, iron, zinc (Zn), nickel, chromium (Cr) and copper. Non-essential heavy metals include lead (Fe), cadmium (Cd), mercury (Hg), aluminum (Al) and arsenic (As) which even at low concentrations cause toxicity (Saleh and Koller 2018).

Food produced on tainted soil or irrigated with dirty water increase metal content and is a significant source of exposure to heavy metals in both animals and humans (Rai 2018). Application of sewage mud and sewage water to the soil over time gradually rise its harmful content of heavy metals, which are then increasingly absorbed by plants and vegetables and move up the food chain endangering the health of both animals and humans (Alengebawy et al. 2021).

Heavy metals present a serious risk in feed and diet and prolonged exposure might have toxicological repercussions for plants, animals, and people due to excessive exposure of metals including cadmium, lead, arsenic, chromium, and mercury (Ali et al. 2019). Both direct and indirect effects on human health are caused by these metals. The grazing animals deposit heavy metals on pasture grasses or forages because ofheavy metal contamination in rural areas caused by the dumping of industrial effluents and sewage sludge (Patel et al. 2019).

Sources of Environmental Contamination

Anthropogenic processes and natural sources contribute to the contamination of the environment due to heavy metals. The heavy metals are emitted naturallyby volcanism, wind erosion, wild fires, and biogenic sources. They are also found in nature as oxides, sulphates, phosphates, hydroxides, sulphidesand organic compounds (Masindiand Muedi 2018).

In agriculture industries, manufacturing of different chemicals like insecticides and pesticides are responsible for the release of arsenic and combustion of fossil fuels release nickel and mercury. Anthrogenic processes are also involved to release heavy metals into the environment through metallurgical processes, residuals of organic matter, and transportation. (Zaynab et al. 2022).

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Health Impact of Heavy Metals

Cadmium Toxicity

The most prevalentelement that occurs naturally is cadmium, which was identified in the early nineteen centuries (Gende and Schmeling 2022). Bivalent, bluish-white, soft, malleable, and ductile cadmium is highly hazardous to humans and other living things. It comes from the cadmium ore greenockite, where it may be found in nature in the form of a mineral (Vaid et al. 2022). Compounds containing cadmium are exceedingly harmful to humans, animals, and plants. The presence of cadmium is widespread in the air, soil, water, plants, and ultimately in animal tissues (Dad et al. 2021). The smelters are main sources where pollution of Cd occurs but burning fossil fuels like coal and burning municipal garbage like plastic and nickel-cadmium batteries are two additional possibilities (Balali-Mood et al. 2021).

Additionally, rain can drop cadmium on the ground, and changing the acidity of the water can make it more soluble in cadmium (Yaciuk et al. 2022). It can easily flow by water movement in the top layers of soil, from where it is absorbed by plants and collected in leafy vegetables, root crops, cereals and grains as a result of which it enters the food chain. In the human body, cadmium has been identified to perform not a single physiological task. Because of this, focus has been drawn to its potential for biohazards. As soon as cadmium is absorbed, it builds up in the body for the rest of one's life. Intoxication with cadmium can damage the kidneys, bones and lungs (Al-Lami et al. 2020). The liver, lung, testicles, and hematological system of animals are among the organs that cadmium poisoning is known to impact (Aliasgharpour2020).

High amounts of cadmium are known to irritate the stomach, which eventually leads to vomiting, pain in the abdomen and diarrhea. Abdominal and muscle cramps, headaches, fatigue, and shock are among the symptoms of acute cadmium consumption that often develop within 30 minutes (Al-Lami et al. 2020). The kidneys, liver and circulatory system are particularly affected, but reproductive tissues and growing embryos have seen the worst side effects (Mizoguchi et al. 2022).

Chromium Toxicity

Vaquelin made the discovery of chromium in 1798 and it is the 21st most common mineral on earth which is present in chromite ore, containing iron and oxygen. The threevalance states of chromium are chromium (0), chromium(III) and chromium (VI) (Majhi et al. 2021). Because chromium (VI) is the most poisonous and carcinogenic of all the metals and has negative effects on the health of people, animals and plants (Mushtaq et al. 2021). Industrial wastes and other sources such as plating, painting, cooling tower fluids and chromate manufacturing are found to have contaminated ground water in the United States (Yoshinagaet al. 2018). Due to the high volume of disposed residual tanning floats, chromium may be viewed as a source of pollution (Younas et al. 2022).

The metallurgical, chemical and refractory sectors are the three largest industries in the world which employ chromium. It is used to make different metal alloys and stainless steel and additionally it is utilized in the creation of joint prosthesis. Additionally, it is utilized in the purification of water, drilling mud, safety matches, copier toner, corrosion inhibitors and photographic chemicals, magnetic tapes and drilling (Laurenti et al. 2017). Cr (VI) is 1000 times more hazardous and 100 times more mutagenic than trivalent chromium (Mushtaq et al. 2022).

Water contamination through water leaching and industrial release is more critical than inhalation of chromium compounds via air emission spread by cigarette smoke, automobile emissions and others (Pavesi and Moreira 2020). Chromium plays an important role in boosting up of insulin for normal glucose metabolism and lipid metabolism and also helps in lowering triglycerides and cholesterol (Prasad 2016).

Chromium toxicity manifests similarly to other heavy metal toxicity in terms of signs and symptoms. Depression, diarrhea (dysentery), dyspnea, paralysis, inappetance, and mortality within a short period of time are some of these symptoms. The lungs, kidneys, and liver experience pathological alterations as a result of Cr poisoning. Chromium exposure severely damages the lungs, leading to mucosal erosion and inflammatory changes in the respiratory tract. Bronchial spasms or even an allergic reaction may occur as a result of chromium poisoning. Hexavalent chromium has also been linked to renal failure, acute renal tubular necrosis, and lung cancer (Deng et al. 2019).

Chromium exposure causes cellular toxicity by generating ROS and cause damage of DNA through activation of high levels of oxidative stress andepigenetic gene expression (DesMarias and Costa 2019). The toxicity and carcinogenicity of Cr depends on time, route and concentration of exposure (Aliasgharpour 2020; Balali-Mood et al.2021).

Nickel toxicity

Nickel occurs naturally in the environment but, in very small amounts. Australia, Canada, Cuba, Indonesia, New Caledonia and Russia are the main countries where nickel ores are found in deposits (Vahed et al. 2021). If nickel is volatile, it is extremely poisonous because it is lipid soluble and can pass through cell membrane. Humans have been discovered to be particularly poisonous to nickel carbonyl (Sule et al. 2020). It is used to create steels that are heat and corrosion resistant. Acute toxicity and immunological problems may result from its usage in the manufacture of nickel salts,the fabrication of NI-CD batteries, the development of moulds for the hollow glass industry, the production of coins, jewelry and medical and dental implants (Al-Lami et al. 2020; Salimi et al. 2020). Animals and plants can become poisonous to nickel if they consume polluted water. Acid rain is by far the main cause of water pollution. Acid rain has the propensity to release nickel from the soil and raise the concentration of nickel in groundwater (Begum et al. 2022). Nickel contamination in water can have negative neurological consequences as well as gastrointestinal problems such as nausea, vomiting, and diarrhea (Pirhadi et al. 2021).

Nickel allergy"—an allergic dermatitis—can be brought on by occupational contact. Due to wearing nickel-plated jewellery, skin rash or allergic dermatitis may develop in cases of dermal contact. Women are more susceptible to the effects of nickel than men, particularly when pregnant women who work in the metallurgical industry have babies with structural defects (Aliasgharpour2020; Rehman et al. 2021).

At the cellular level, it can result in DNA strand breaks, cross-linking of DNA protection, DNA oxidation, nucleotide removal, gene mutations, changes of chromatids, binding to enzymes involved in DNA repair and protein degradation, formation of ROS, enhancement of lipid peroxidation, altering calcium, and sulfhydryl balance, and glutathione degradation (Das et al. 2019).

Lead toxicity

Lead is a bluish-grey metal that occurs naturally and is found in the earth's crust in small amounts (Khatun et al. 2022). It is found in rocks, soil, plants and animals. It is a ubiquitous element, which occurs at a pretty low level (Rahman and Singh 2019). Lead has been used in industry, household products, cigarettes, ceramic glazes, paint, smelters, televisions, pesticides, computer monitors, batteries, explosives, pipes and toys for centuries (Sanz et al. 2022).

Because it can result in significant environmental contamination and health issues, lead is the most significant toxic heavy metal in the environment (Aliasgharpour2020). Industrial processes like burning fossil fuels, mining, smelting, manufacturing, and recycling are the main causes of environmental contamination. (Marzilli 2019).

The chemical or heavy metal pollution which has caused toxic, mutagenic, or carcinogenic effects on human health has risen due to the combination of new chemical substances (Alengebawy et al. 2021). The noxiousness of lead was recognized in 200 B.C. The fact that lead causes anemia and colic in human beings was discovered in 250 B.C (Pereira et al. 2020).

The materials used in lead pipes, tin soldiers, and brass fixtures are the primary source of lead in drinking water (Chang et al. 2022). One of the sources of lead in the air, water and soil that ultimately enters the food chain is found in phosphate rocks (Kumar et al. 2019).

Lead produces lung disorders, anemia, liver damage, renal impairment and also affects nervous system by exposure to high concentration in adults and children (Singh et al. 2018). General symptoms of lead poisoning in children due to increase in lead levels in blood include abdominal pain, irritability, lethargy, anorexia, pallor body, ataxia and slurred speech (Rees and Fuller 2020). Encephalopathy and gastroenteritis are frequently seen in dairy cattle as a result of the animals grazing on contaminated forages caused by the application of sewage sludge to the pastures (Gupta et al. 2021).

High amounts of this heavy metal in the blood during pregnancy have been proven to allow it to breach the placental barrier, which can result in fetal abnormalities like low IQ, encephalopathy, neurological diseases, and disruptions in calcium levels in nerve cells. Lead exposure during pregnancy can cause miscarriage, early birth, low birth weight and stillbirth (Aliasgharpour 2020; Rehmanet al. 2021).

After ingestion, 99% of the lead is bound to hemoglobin and circulates through the circulatory system to soft tissues, bones, liver, kidneys, and hair, which is retained primarily in teeth and bones (where it is absorbed into the mineral in place of calcium). Particularly when a woman is pregnant, the bloodstream can be reintroduced with accumulated lead which exposes the foetus (Cioseket al. 2021).

Arsenic toxicity

Arsenic is among the most toxic metals found in the environment and is found in metallic, metalloid, inorganic, and organic form. Arsenic exist in three valence states: As (0), (III) and (VI). In general, the inorganic form of as is more toxic than organic form (Thakur et al. 2021; Genchi et al. 2022).

It is omnipresent metal found in air, land, ocean, lakes, and water (Rahamanet al. 2021). Arsine gas is a very poisonous substance. Mining, metallurgical and microelectronics sectors all produce arsine gas. Ground water contamination, which is a severe issue, is also discovered to contain arsenic. This is particularly true in the third world nations (Ali et al. 2019). More than 100 million people worldwide are at risk of mortality from arsenic poisoning from the food and drinking supplies (Nurchiet al. 2020).

Cancers of the kidney, liver, lungs, and bladder are brought on by prolonged exposure to arsenic (Aliasgharpour2020; Rehman et al. 2021). Abrupt arsenic exposure can result in a variety of symptoms, including tachycardia, acute encephalopathy, congestive heart failure, stupor, convulsions, paralysis, coma, and even death. Arsenic exposure causes peripheral neuropathy, cardiovascular discomfort, peripheral vascular disease, kidney and liver damage, hypertension, myocardial infarction, and leukopenia (Fakhar et al. 2022). Arsenic's long-term side effects include skin deformities, neurotoxicity effects, severe pulmonary illnesses, insanity, cognitive impairment, loss of hearing, and coronary heart disease (Singh et al. 2022).

Table 1: List of five key metal's effects on human health

Metals	Deficiency health effects	Toxicity health effects	References
Iron (Fe)	Anemia Mental retardation Brain damage	Cardiac toxicity Metabolic disorders Hepatotoxicity Neurodegenerative disorders Cancer	Engwa et al. 2019
Selenium (Se)	Kashin disease	Skin rash Loss of hair Neurologic disorders Fatigue Abdominal pain	Vinceti et al. 2018
Iodine (I)	Neutropenia Osteoporosis Impaired mental confusion Hypothyroidism	Hypothyroidism Goiter Hyperthyroidism	Niziński et al. 2021
Copper (Cu)	Neurological disorders Neutropenia Osteoporosis Hypopigmentation	Brain damage Necrosis Liver cell erosion Death	Rahman and Singh 2019
Zinc (Zn)	Abdominal disturbance Behavioral changes Growth retardation Immunological deficiencies	Nausea, vomiting and diarrhea Lethargy Fever Anemia	Chasapis et al. 2020

At the cellular level, arsenic compounds have the capacity to bind specific structures or substitute specific substances. They can also inhibit the mitochondrial enzymes involved in cellular respiration. It inactivate particular enzymes like thiolaseand dihydrolipoyl dehydrogenase and disrupt oxidative phosphorylation (Bahrami et al. 2020).

Mercury toxicity

Mercury is a metallic, shiny, odorless sliver white fluid, which upon heating converts into dull and colorless gas. Mercury is both extraordinarily deadly and bio-accumulative (Kumar and Kumar 2019). Mercury enters the climate through a typical breakdown of minerals present in rocks, which are moved to the soil via air and water. The centralization of mercury in the climate is expanding step by step, and this is exposed to human action. The majority of the mercury is delivered into the air through fossil fuel combustion, mining, refining, and strong waste burning (Charvát et al. 2020).

Mercury in all chemical forms has capability to cross the placental, and blood-brain barriers, and also secreted in milk (Pajewska-Szmytet al. 2019). Exposure of mercury causes the toxicity associated with fever, insomnia, tremors, weight loss, and excessive perspiration (Bharti and Sharma 2021). The reproductive system's ability to cause infertility in both men and women was proved by the research. The spermatogenesis of males is impacted, while progesterone and estrogen levels in females may be disturbed, resulting in ovaries that don't function normally, irregular menstruation, and a uterus that slopes (Balali-Mood et al. 2021).

Mercury genotoxicity was linked to DNA damage, conformational changes in DNA repaired proteins, genetic mutations, mitotic spindle, chromosomal segregation, and effects on nucleic acids (Gashi 2018). Moreover, for informative survey analysis, seafood is a core source of mercury contamination. Food products prepared from fishmeal for livestock and eventually for humans have great chances of septic conditions and ultimately cause toxicity (González-Montaña et al. 2019).

Other essential metals

The majority of necessary metals function as enzyme catalysts and can be hazardous in excessive concentrations. Metals frequently combine with organic substances including amino acids, proteins, and peptides in tissues and bodily fluids to produce complexes.

Table 1 shows the list of five key metals which show serious effects on the human health.

Conclusion

Heavy metal toxicity and its effects on the ecosystem are a global problem due to their transportation through the air, soil and water. The potential of ingesting heavy metals through food, air, and drinking water depends on a number of variables, including concentration and numerous primary sources. Humans need these metals in trace amounts for normal cellular, metabolic, and hormonal function; nevertheless, if the limit is exceeded, it can have serious negative health impacts. The toxicity of these metals has a significant negative impact on the soil by destroying microorganisms that are essential to improve soil fertility and nutrition.Much emphasis has been paid to their removal from aquatic and soil environments.

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Malnutrition in Children of Growing Age and the Associated Health Concerns

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INTRODUCTION

Malnutrition is characterized as the insufficient or exclusive consumption of a dietary nutrient by children in particular. Adolescence under-nutrition (wasting, hindering, and underweight), micronutrient deficiencies, as well as overweight and stoutness, constitute a triple weight of illness, especially for low- and middle-income countries, and it is one of the main causes of chronic insecurity and a significant barrier to self-awareness and the achievement of full human potential globally (Kalu and Etim 2018). The malnutrition of children (under-nutrition) and the prevalence of non-communicable diseases (NCDs) in adults are major public health issues in the world today. Despite convincing evidence connecting prenatal malnutrition with NCDs, little is known about long-term health outcomes (Grey et al. 2021). From 10–11 million children under the age of five, over 50% dies each year from preventable causes as a result of malnutrition, which is a significant public health issue in the developing world (Collins et al. 2006). Children's deaths are primarily caused by malnutrition worldwide. Community-based interventions for malnutrition require the use of universally validated screening tools and the implementation of appropriate interventions depending on the severity of malnutrition (Khan et al. 2016). Malnutrition is more prevalent in rural areas in India because of inadequate diets, recurrent infections, lack of proper care, and unequal distribution of food within the family. A child's rapid growth and development require optimal nutrition during his or her infancy and early childhood. Deficiencies in nutrients lead to lower immune defenses against diseases and recurrent infections result in nutrient deficiencies. This adversely affects the mental and physical development of children as well as their learning capacity in the future (De and Chattopadhyay 2019).

Epidemiology of Childhood Malnutrition

Of all the developing nations, Pakistan has one of the highest rates of child malnutrition. Around 165 million children under the age of five are malnourished worldwide. At least half of all child fatalities are caused by malnutrition. Developing and underdeveloped nations are typically the only places where this issue is prevalent causing high morbidity and mortality in children's. The cause of about half of all child deaths worldwide is under-nutrition. According to the National Nutrition Survey, 50% of children were anemic, 33% of children were underweight, approximately 44% were stunted and 15% were wasted (iron deficiency). Comparing Pakistan to other emerging nations, there has been a slight decline in the prevalence of child malnutrition during the past two decades (Asim and Nawaz 2018). Children who are malnourished suffer from a host of factors including poverty, poor sanitation, crowding, infectious diseases, depression, and abuse (Awoyemi et al. 2012). Many nations in the era of the Millennium Development Goals succeeded in reducing the rate of childhood under nourish, but there was uneven improvement. The majority of Asian countries saw dramatic reductions, but progress in Africa was less encouraging, and the overall number of stunted children increased as a result of population expansion. Globally, this improvement hit a wall about 2014 and is currently regressing, as noted in the latest study by the State of Food Security and Nutrition in the World 2019. Around 51 million children under the age of 5 were wasted (too light for their height) and 151 million were stunted in the world in 2017, with poor nutrition being the primary proximal cause. However, it was stated that 38 million people were fat (Mwangome and Prentice 2019).

Prevalence

Globally and particularly in Nigeria malnutrition is measured by three anthropometric factors for stunting, wasting, and underweight and it is a serious health issue for newborns, toddlers, and preschool-aged children. Nigeria is one of the top 10 nations for children under five that are malnourished. The frequency of stunting, wasting, underweight, and obesity in children is 37%, 7%, 22%, and 2%, respectively, in Nigeria (Folayan et al. 2020).

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The number of stunted and wasted children in the world is around 155 million, indicating a high incidence of malnutrition. According to estimates, under-nutrition accounts for 45% of all fatalities in children under the age of five around the world. The frequency of underweight children worldwide was reported to have decreased from 25% in 1990 to 15% in 2015 around the end of the millennium development goals (MDGs) era. Because 90% of the underweight infants were discovered in Africa, notably in the Sub-Saharan region, this drop was regretfully not uniformly distributed. Between 1990 and 2013, the proportion of stunted children in Sub-Saharan Africa actually grew to over one-third (Adebisi et al. 2019). The Democratic Republic of the Congo (DRC) is one of five nations in the world with a high mortality rate for children under five (India, China Nigeria and Pakistan) and malnutrition is one of the main causes of death in these nations, along with other illness like diarrhea, pneumonia, and malaria, which are more prevalent in young children (Mukuku et al. 2019).

Types of Malnutrition

According to World Health Organization (WHO), malnutrition divided into three groups including; micronutrient related malnutrition, over-nutrition and under-nutrition (Khaliq et al. 2022). Under-weight, stunting and wasting are three types of under-nutrition. Obesity is mainly related to the over-nutrition. Micronutrient are further classified as micronutrient related malnutrition (MRM) deficiency and MRM overload (Khaliq et al. 2021; Khaliq et al. 2022).

A- Under-nutrition

Under-nutrition has intense effect on growth, development and survival of children. Globally 200 million children under five face stunting and wasting (Keeley et al. 2019). Under-nutrition is defined as the weakening, stunting, or micronutrient deficiencies (such as anemia, zinc, and vitamin A). Stunting and wasting both point to distinct nutritional deficit issues. Wasting (low weight for height) is a kind of acute malnutrition characterized by inadequate dietary intake to satisfy protein and energy requirements. Infectious disease makes wastage more prevalent in underdeveloped nations with long-term or short-term food insecurity (Abdullah 2015). Stunting, wasting, and underweight were all prevalent at varying degrees (10.4%, 5.3%, and 18.4%) (Boah et al. 2019).

i- Under-weight

Underweight is described as population of children whose weight is less as per their age (Asim and Nawaz 2018). Particularly in underdeveloped nations, underweight is a substantial risk factor for infection in children and teens, perhaps due to hunger and less hygiene standards. Not only underdeveloped but also in Western nations underweight children are more susceptible to illness. Underweight children were more likely to be admitted to emergency rooms in the USA than normal-weight, overweight, or obese children, according to an observational chart assessment of the data.

ii- Stunting

Stunting is described as population of children who are too short for their height (Keeley et al. 2019). Stunting in childhood increased the risk of infectious diseases, poor cognitive development, mortality rate, poor productivity and non- communicable diseases in adulthood (Kang and Kim 2019). 149 million children are globally stunted and 1 in 3 children is stunted in Asia and Sub-Saharan Africa. The cause of stunting start from the nutritional status of mother. Mothers who are stunted in childhood are at high chance to having stunted child (Keeley et al. 2019).

iii- Wasting

Wasting is defined as a child who is too thin for his or her height (Keeley et al. 2019). Wasting is due to improper dietary intake and disease. Wasted children have increased risk of death therefore they require urgent nutritional or medical help. Wasting in growing age cause delayed physical development and weakened immunity (Donkor et al. 2022). Globally 49.5 million children are wasted and in South Asia 1 in 7 children under five is wasted (Keeley et al. 2019).

B- Over-nutrition

Over-nutrition leads to over-weight and obesity in children that affect both early and later life of children (Keeley et al. 2019). Obesity is a global challenge, and the prevalence of obesity and over-weight is increasing in low and middle income countries as compared to high income countries (Adeomi et al. 2022). Globally 40.1 million children are over-weight, and in Eastern Europe and Central Asia 1 in 7 children under five is over-weight (Keeley et al. 2019). In over-weight children, the health outcomes are inferior then adults. It increase the risk of non-communicable diseases such as insulin resistance and heart diseases in early life, and 41 percent of cancer chances increased in obese children (Pant and Vaidya 2018).

C- Micronutrient Related Malnutrition

Humans who are deficient in vital vitamins and minerals, commonly referred as micronutrients, are said to have "hidden hunger." Nearly one-third of the world's population, or two billion people, suffer from micronutrient deficiencies

(Harding et al. 2018). When extended, insufficient dietary intake of macronutrients (fat, protein, and carbs) and micronutrients (vitamins and minerals) can have negative consequences on health outcomes and possibly start a cycle of poor health (Eggersdorfer et al. 2018). Globally, hidden hunger affects a sizable population and has a variety of negative consequences, such as poor health, stunted growth, low productivity, mental impairment, and untimely death (Ekholuenetale et al. 2020). Iodine deficiency (ID), the world's leading contributor to children's preventable brain damage and mental retardation, needs top priority among micronutrient deficiencies (Asfaw and Belachew 2020).

Causes of Malnutrition

There is multiple causes of malnutrition in growing age including; diseases, nutritional status, inadequate dietary intake, inadequate child care, household food insecurity and insufficient health services. These all causes belong to the potential sources like economic and organization sources (Mkhize and Sibanda 2020).

Factors Related to Malnutrition in Growing Age

Malnutrition is a major problem of under-developed and developing countries. Inadequate diet cause malnutrition in growing age like undernutrition, fatigue, multivitamins deficiency and feeling of weakness that all impact on child growth (Shahid et al. 2021). There are many factors responsible for malnutrition and the basic factors are.

i- Inadequate Diet

The issue of inadequate diet usually occurs in growing children (Achakzai and Khan 2016; Shahid et al. 2021). Maternal factor like BMI of mother is also a risk factor for child malnutrition (Khan et al. 2019). Inadequate feeding habits are a major cause of the emergence of malnutrition in young infants (Eshete et al. 2018). The increased nutritional requirements of pregnancy and fetal growth place women of reproductive age who live in under-resourced environments in low- and middle-income nations at an especially high risk of receiving insufficient nutrients, especially of important micronutrients (Lander et al. 2019).

ii- Food Insecurity

Food insecurity is also a principal factor of child malnutrition in South Africa. Food insecurity effect the child nutrition because of the consumption of less or improper diet. Consumption of unhealthy and less diet also have adverse effect on child nutrition (Ntila et al. 2017; Mkhize and Sibanda 2020).

iii- Low Household Income

Low household income effects the nutritional status of children which lead to malnutrition. According to national survey in south Africa, there are estimated 2.5 billion children, under age of five year are belong to poor families or low income household (Mkhize and Sibanda 2020). Having low income possess some families to live in the house with poor substructure and unhygienic conditions which lead to different infections that effect people especially children (Guevara-Romero et al. 2021).

iv- Family Size

The increase number of family size effect the income and education of children and the lack of sanitary condition like drinking water and sewerage affect the health. Health problems are directly proportional to the malnutrition of children at growing age which causes retarded growth, negative effect on body size and growth and also effect the mental health (Cedeño et al. 2022; Calceto-Garavito et al. 2019).

v- Environmental Factor

Unimproved water, poor sanitation, and biomass fuel are the second most significant worldwide attributable burdens as causes of child stunting. A study of 137 developing nations found that unimproved sanitation was responsible for an estimated 7.2 million instances of stunting (Amare et al. 2019).

vi- Educational Status

Parental education is a significant predictor of children's nutritional status. The higher parental educational status is connected with better child parenting and wellness. Children with tertiary-educated parents are more likely to have a nutritious diet, regardless of economic level, due to their greater level of understanding on fundamental child nutrition. Parents with higher educational attainment may encourage values that are harmful to their children's health (Kalu and Etim 2018).

How Maternal Malnutrition Affect Children

The mother is a kid or infant's primary caregiver. An infant's health and wellbeing are somewhat reliant on the mother's health and wellbeing (Amaha and Woldeamanuel 2021). It has been demonstrated that infant and mother health are related to a child's birth weight, which indicates that these factors may determine maternal and infant mortality before, during and after birth (Mishu et al. 2020; Ipadeola et al. 2013). Malnutrition, which includes low birth weight, underweight, wasting, stunting, and a lack of micronutrients, affects millions of children and women worldwide (Roy

2018). More than 20% of women are undernourished in various South Asian and sub-Saharan African nations (BMI 18.5). More than 10% of women are under 145 cm in height in various South-Central Asian nations. However, anemia affects 30% of reproductive-age women globally (Ravaoarisoa et al. 2018). Women who are pregnant or breastfeeding are at danger and the newborn babies will be more susceptible to diseases in later life. In infancy and adulthood, behavioural and cognitive impairment caused by maternal and newborn malnutrition has been linked to increased susceptibility to neuropsychiatric illnesses (Sinha et al. 2018). The effects of infectious diseases on maternal, fetus, neonatal, and child health are more significant and maternal malnutrition aggravate this effect (Blakstad and Smith 2020).

Diagnosis of Malnutrition in Children

The human body may be measured quantitatively without any physical contact. The Centers for Disease Control and Prevention (CDC) claim that anthropometry is a useful tool for determining both children's and adults' nutritional health (Casadei and Kiel 2019). An individual's nutritional status is now considered to be highly affected by anthropometric measures. Adverse changes in the body composition result from malnutrition, either under-nutrition or over-nutrition. Malnutrition can lead to increased morbidity or death if the body's energy reserves are severely depleted. Changes in the status of nutrition may be easily assessed using the simple and efficient approach of anthropometry. Additionally, it offers a way to assess if nutritional treatment is being utilized appropriately (Suriah et al. 1998). The fundamental measurements of anthropometry include height, weight, head circumference, body mass index (BMI), waist, hip, and thigh circumferences to measure adiposity, and skinfold thickness (Casadei and Kiel 2019).

Body Mass Index (BMI)

WHO claims that BMI is a basic measure of weight in relation to height that is often used to diagnose humans as being underweight, overweight, or obese. Its formula is kg/m^2, or weight in kilograms divided by height in meters squared (Naeem et al. 2021).

Associated Health Risks in Malnutrition

Malnutrition at any stage of childhood affects schooling and thus a child's lifetime earning potential. It impacts educational outcomes, including reduced learning capacity (either as a result of early cognitive deficits or reduced attention span) and reduced overall years of schooling. A triple burden of disease is caused by all types of childhood malnutrition, including under-nutrition (wasting, stunting, and underweight), micronutrient deficiencies, as well as overweight and obesity. Childhood malnutrition may have long-term, irreversible impacts, such as stunted physical growth and delayed cognitive development. Additionally, inadequate nutrition might impair sensory-motor development, reproductive health, and children's susceptibility to infections and hereditary illnesses like diabetes. In addition, under-nutrition increases health care expenses, lowers adult productivity, and stunts economic growth, which can lead to a protracted cycle of illness and poverty. Most cases of childhood under-nutrition occur in low- and middle-income countries, primarily as a result of poverty, which is linked to poor feeding practices, unsanitary living circumstances, and a lack of access to health care (Vassilakou 2021). The summarized health risk of malnutrition in growing children is enlisted Table 1.

Protein Energy Malnutrition (PEM)

Protein-energy malnutrition (PEM) is a common childhood disorder and it is commonly caused by deficiency of micronutrients protein and energy. PEM manifests as underweight (low body weight compared with healthy children), stunting (poor body growth), wasting (acute weight loss), or edematous malnutrition (kwashiorkor) (Ahmed et al. 2020). PEM has been named by the WHO as one of the main issues affecting children worldwide. Additionally, it has been discovered over time that PEM in children, particularly at the most critical stage of their development, is a problem in developing nations like India. Additionally, this is a problem that primarily affects rural populations and the poor and is brought on by problems with poverty, poor environmental sanitation, poor dietary practices, low socioeconomic status, maternal education, frequent infection, inadequate household food security, high rice consumption, frequent acute illness, and low birth weight of children (Satapathy et al. 2021).

Primary Acute Malnutrition

Primary acute malnutrition affects children and is particularly prevalent in low- and middle-income countries. It results from insufficient food availability brought on by socioeconomic, political, and environmental causes. The contributing causes of primary acute malnutrition include household food insecurity, poverty, inadequate prenatal nutrition, intrauterine growth restriction, low birth weight, inadequate breastfeeding, frequent infectious diseases, poor water quality and poor hygiene (Dipasquale et al. 2020).

Secondary Acute Malnutrition (SAM)

According to the United Nations Children's Fund, World Health Organization, and World Bank Group,2017, approximately 17 million children are suffering from severe acute malnutrition (SAM) worldwide, which is indicated by

Table 1: Health risks of malnutrition in growing children

Health risk	Causes	Effects	References
Protein energy malnutrition	Deficiency of micronutrients protein and energy	Stunting Wasting	(Ahmed et al. 2020; Satapathy et al. 2021)
Primary acute malnutrition	Insufficient food availability Poverty	Low birth weight Infectious diseases	(Dipasquale et al. 2020)
Kwashiorkor	Extreme protein deficiency	Swelling in both extremities	(Benjamin and Lappin 2021)
Marasmus	Insufficient energy intake	Children feels weak and lethargic Hypotension, and hypothermia bradycardia	(Pham et al. 2021; Dipasquale et al. 2020)
Iron deficiency anemia	Iron deficiency	Maternal anemia is linked to low birth weight, higher perinatal mortality	(Cusick et al. 2018; Bhadra and Deb 2020; Mantadakis et al. 2020)
Scurvy Rickets Pellagra Beriberi	Vitamin deficiencies	Periodontitis Enamel, hypoplasia Defective tooth development Oral epithelial development Osteomalacia	(Antonucci et al. 2018; Gossweiler and Martinez-Mier 2020)
Obesity	Excess energy	Hypertension Type -2 diabetes	(Oriaifo et al. 2019; Seneviratne and Rajindrajith 2022)
Congenital heart disease	Low energy intake Protein losing	Vomiting and, have trouble eating Vocal cord dysfunction Chylothorax	(Larson-Nath and Goday 2019; Herridge et al. 2021; Shahid et al. 2021)

a mid-upper arm circumference (MUAC) less than 115 mm or a weight-for-height z-score (WHZ) less than 3 SD (Stobaugh et al. 2019). A set of recommendations for treating severe malnutrition in children were released by the World Health Organization (WHO) in 1999. Mortality dropped from 30% to 5% in nutritional centers all over the world after the application of this approach. An urgent issue around the world is providing adequate care for sick and malnourished children (Bernal et al. 2008).

Kwashiorkor

It is a condition characterized by extreme protein deficiency and swelling in both extremities. Infants and toddlers are typically affected, most frequently between the ages of weaning at 5.This is a severe form of protein-energy malnutrition characterized by a lack of protein and edema in both extremities. Extremely severe cases of malnutrition and areas of extreme poverty around the world are affected by the disease (Benjamin and Lappin 2021). Edema in kwashiorkor resolves with dietary therapy before the plasma albumin concentration increases.It has also been proposed that hypoalbuminaemia is not included in the etiology of the condition (Coulthard 2015).

Marasmus

The word "marasmus" is a Greek word meaning to waste or wilted. The most typical sign of acute malnutrition is marasmus. Marasmus, also known as phthisis, dystrophy, and cachexia, has been reported for the longest as the usual type of severe malnutrition (Pham et al. 2021). It results from insufficient energy intake over several months to years. It is characterized by wasting of body tissues, especially muscles and subcutaneous fat, and typically results from extreme limits in calorie intake. It is the body's physiological adaptive reaction to hunger in response to severe loss of energy and all nutrients. The children feels weak and lethargic, and have associated, hypotension, and hypothermia bradycardia (Dipasquale et al. 2020).

Children at Risk of Iron Deficiency Anemia

Children are at risk for iron deficiency at three different ages: late prenatal and neonatal, 6 to 24 months, and adolescence. The first 1000 days, sometimes referred to as early-life iron deficiency, are when the brain grows and develops quickly, and they can have a detrimental effect on neurodevelopment. Around two billion individuals worldwide suffer from iron deficiency and iron-deficiency anemia (IDA), with the majority living in low- and middle-income nations. Malaria and parasite infections, other dietary deficiencies, chronic illnesses, hemoglobinopathies and lead poisoning are additional causes of anemia in these countries (Mantadakis et al. 2020).

Maternal iron deficiency anemia, preterm birth, and prenatal problems including intrauterine growth restriction, gestational diabetes mellitus, maternal smoking, maternal obesity, and inflammation are the most common causes of iron deficiency in the fetal and newborn period. The most frequent causes of iron deficiency in people are eating a diet low in iron and/or containing iron binders, persistent gastrointestinal blood loss brought on by an aversion to cow's milk or a hookworm infection, and anemia (Cusick et al. 2018). Maternal anemia is linked to low birth weight, higher perinatal mortality, and lower labor productivity in countries with limited resources. In these conditions, it might be difficult to maintain a healthy iron balance because animal-based meals with high iron bioavailability tend to be expensive or hard to come by. Other significant risk factors for IDA in low-income nations include poor vitamin C intake, diets high in iron absorption inhibitors, and infrequent meat eating. Using micronutrient powders to fortify complementary foods at home with iron has been shown to significantly lower the risk of iron deficiency anemia (IDA) in infants and young children in developing nations, but it is also linked to unfavorable changes in gut flora and the induction of intestinal inflammation, which can cause diarrhoea and hospitalization. Infants older than six months of age, adolescents with excessive menstrual bleeding, women of childbearing age, and elderly persons are more likely to have IDA (Mantadakis et al. 2020).

Lack of vitamin B12 (riboflavin) can cause anemia to develop. It has been demonstrated that riboflavin supplements given alongside iron supplements have a stronger impact on haemoglobin concentration than iron supplements alone. There have been cases of riboflavin deficiency in school children, newborns, pregnant women, and breastfeeding mothers. Vitamin C influences iron metabolism and improves non-heme iron absorption. Pregnant women, children who only consume cow's milk, the elderly and smokers are among the groups at risk for vitamin C insufficiency. Children and non-pregnant women who take vitamin C supplements had higher haemoglobin concentrations and serum ferritin levels (Bhadra and Deb 2020).

Vitamin Deficiencies in Children

Vitamins are necessary organic substances that speed up metabolic processes. Additionally, they serve as antioxidants, electron donors, or transcription effectors. They can be derived from food and supplements, or in rare situations, our bodies or gut bacteria can produce them on their own. Systemic effects from severe vitamin deficiency include the emergence of scurvy, rickets, pellagra, and beriberi. Oral problems can also be caused by some mild, moderate, and severe deficits. Vitamin A deficiency has been linked to periodontitis, enamel hypoplasia, defective tooth development, and oral epithelial development (Gossweiler and Martinez-Mier 2020).

It is well recognized that a vitamin D deficiency leads to osteomalacia in adults and rickets in youngsters. Nutritional rickets almost vanished from developed nations after vitamin D deficiency was identified and food supplements with vitamin D were introduced. However, over the past two decades, a number of circumstances have caused the disease to resurface, with non-Caucasian children accounting for an increasing number of cases. In wealthy nations, hypovitaminosis D in children is an emerging public health issue. The emergence of vitamin D insufficiency may be favoured by new lifestyle patterns, the current "epidemics" of obesity in children and adolescents around the world, and other preventable risk factors. Hypovitaminosis D has been linked to the development of major extra-skeletal health issues in children, such as atopy and autoimmune disorders, in addition to its effects on the skeleton (Antonucci et al. 2018).

Vitamin Deficiencies Linked to Childhood Obesity

The major issue occurring worldwide is childhood obesity. Overweight during infancy and adolescence is a well-known risk factor for adult obesity as well as the emergence of comorbidities. In relation to vitamin D (vit D), its role in immunological processes and lowering the risk of chronic illnesses have been taken into account in addition to its impact on bone health and calcium and phosphorus metabolism. A study with the general pediatric population of the United States of America, aged 1 to 11 years found that the prevalence of vitamin D deficiency varies by geographic region and is estimated to be 15%. In a cross-sectional survey of a representative sample of adolescents from America aged between 12 to 19, a prevalence of 14% was also discovered (Fiamenghi and Mello 2021).

Over-nutrition or Obesity in Children

The term "obesity" describes an individual who has an excessive buildup of body fat that is dangerous to their health. An imbalance between calorie consumption and expenditure is the primary driver of obesity, with excess energy being retained as fat in adipose tissue (Seneviratne and Rajindrajith 2022). Including young children, the obesity epidemic has affected every age group(Perng et al. 2019). In developing countries, a number of factors contribute to the rise of childhood obesity including reduced exercise, higher calorie consumption, high socioeconomic position, urbanization and living in large cities, socio cultural influences, age, female gender and school food programs (Arfines et al. 2020). Obesity is linked to problems that might develop in childhood and adolescence and last into adulthood. Hypertension is one of these problems, which increases the risk of long-term cardiovascular illnesses and early mortality. According to the studies in Nigeria among school-aged children, the obese children had a considerably greater proportion of children with blood pressure in the pre-hypertension and hypertension range (Oriaifo et al. 2019). One of the key factors contributing to a poor pregnancy outcome is maternal obesity or over-nutrition. Obesity during pregnancy has a severe impact on fetal and embryonic development, but it may also have long-term effects on the children's health (Sarker et al. 2019). The primary cause of type 2 diabetes is obesity (Seneviratne and Rajindrajith 2022). Maternal obesity during pregnancy and lactation affects offspring's neurodevelopment and raises the risk of behavioral and emotional issues like hyperactivity, autism spectrum disorder, anxiety, and cognitive impairment. This is supported by growing evidence from epigenetic, clinical, and preclinical studies (Hasebe et al. 2021).

Table 2: Preventive measures/interventions of malnutrition

Stages of life	Interventions for infants or young children	Intervention for mother	References
Mother during pregnancy	N/A	Healthy maternal diet promotion and assistance; provision of food or micronutrients in situations when there is a lack of access to food, Supplementing with iron and folate	(Bhutta et al. 2008)
Lactating mother and child (0-4 months)	Supplementing of food or micronutrients for infants under 24 months	Promoting the best breastfeeding and supplementary feeding methods	(Hawkes et al. 2020)
Children aged ≤ 5	Monitoring and promoting growth and acute malnutrition detection and care Hygienic measures such as hand washing		(Hawkes et al. 2020;Bhutta et al. 2008)

Congenital Heart Disease in Children

One of the most prevalent birth defects is congenital heart disease (CHD) which has a significant morbidity and death rate, particularly in young children who are chronically undernourished (Shahid et al. 2021). The congenital heart disease (CHD) is a dangerous condition affecting on the health of children. According to studies, congenital cardiac problems cause more newborn mortality than the sum of all other congenital abnormalities. Congenital heart disease patients typically have early de-compensation. Heart failure strikes 20% of these kids with in the first week of life, 18% within the first and fourth weeks, and 20% within the first year (Ismail et al. 2021). Children with congenital heart disease frequently experience vomiting, have trouble eating, and can even develop an oral aversion. Regular feed interruptions, vocal cord dysfunction, and chylothorax are postoperative risk factors for malnutrition in infants with congenital heart disease. Children with single ventricle illness also run the risk of developing a protein-losing enteropathy (Larson-Nath and Goday 2019). Congenital heart disease induced a severe impairment in the circulatory system's structure or activity, which consequently had an impact on the respiratory system's performance (Rahayuningsih et al. 2021). Delayed in weight gain, length, and head circumference are indicators of growth challenges faced by CHD children. Lower weight-for-age and weight-for-length are indicators of acute malnutrition. There is reduction in growth velocity, which significantly slows down weight achievement (Herridge et al. 2021).

Prevention of Malnutrition in Children

The timely growth and maturity of physiological, physical, neurocognitive, emotional, and social functions is a sign of good health in children. The design and function of organs and tissues as well as metabolic functions can be irreversibly altered by insufficient intake of energy and nutrients throughout development (Owino et al. 2019). Nutritional status in women during pregnancy and breastfeeding, as well as in newborns and young children throughout the early years of life, has a significant impact on malnutrition in all of its forms during the course of a person's life. Infants who get insufficient amounts of nutrients early in life not only suffer from under-nutrition, but are also more likely to have a more centrally located body fat distribution and they should go on to put on weight in the future and have adverse effects on child's health (Hawkes et al. 2020).

The fundamental preventive health measures used to combat undernourishment at various stages of life that are provided by medical institutions and networks of community-based health professionals are enlisted in Table 2.

These are some interventions in the health system to support and promote nutrition for pregnant women and young children during the first 1000 days (Hawkes et al. 2020) .Children's anemia, iron-deficiency anemia, and iron deficiency can all be improved with the use of micronutrient powders for point-of-use fortification of supplemental meals (Heidkamp et al. 2021). Exercise is advised not just to help with weight reduction and maintenance while treating obesity, but also to improve whole-body insulin sensitivity and both mothers' and children's metabolic profiles (Castro-Rodríguez et al. 2020). The six essential nutrients—carbohydrates, fats, proteins, vitamins, minerals, and water—must be included in the balanced diet in the right amounts and qualities (Ahmed et al. 2019).

Conclusion

Malnutrition is a significant issue in growing children which are contributing in malfunctioning of child growth and the associated health concerns like congenital heart disease, stunting, wasting and vitamin deficiencies. Malnutrition is usually assessed by anthropometric measurements. A child's rapid growth and development require optimal nutrition during his or her infancy and early childhood.

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An Overview of Copper Toxicity and Public Health Concerns with Mitigation Strategies

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INTRODUCTION

Copper is considered as a trace element found on the planet Earth (Dai et al. 2020). It denoted by Cu (from latin: *cuprum*), having atomic No. 29. It is malleable, ductile and soft metal having high electrical and thermal conductivity. Fresh color of copper is pinkish orange. It is commonly used in building material, metal alloys, coins, jewelry, marine hardware, thermocouples for measurement of temperature and in strain gauges (Mehta and Badheka 2016).

Copper is used in electrical wire, plumbing, cooking utensils, coins, jewelry and decorative objects (Thompson 2007). It is also used in agriculture and veterinary medicine as copper salt. Supplementation of copper obtained from both inorganic and organic source have the positive effects in poultry yield (Das et al. 2010). It is also used as growth promoter if fed at the rate of 250 ppm, but supplementation at high quantity leads to decreased in feed intake, poor FCR and depressed growth in broiler (Rahman et al. 2001).

Copper may contaminate the environment when it accumulates abundantly in environment producing many hazards (Hefnawy and El-khaiat 2015; Dai et al. 2020). It is a one of major cause of environment pollution (Ray et al. 2006). Copper is essential part of many enzymes, which are necessary for the normal biological mechanism of body (Gaetke and Chow 2003). It is present in many cells of body but its highest quantity present in brain and liver. It is present in biological system as cupric form (Cu++), but in copper containing enzyme, it is present as bound cation form. Copper is required for all living organism for normal biochemical process (Gaetke et al. 2014). It is a part of many enzymes as a cofactor like metalloenzyme which include lysyl oxide, ceruloplasmin, cytochrome oxidase, superoxide dismutase and tyrosinase. It is also required for free radical defense, cellular respiration, tissue biosynthesis and neurotransmitter functions (Hefnawy and El-khaiat 2015). It also possesses antioxidant activity. It is used in cytochrome copper oxidase and C oxidase as prosthetic group. It is essential part of enzyme as is required for the conformation and integrity of enzyme. It is required for oxidation reduction reaction (Dai et al. 2020). Lysyl oxidase is Cu enzyme which is vital for cross linkage of collagen and elastin. These are required for connective tissue formation. Protein having Cu like ferroxidase and ceruloplasmin transfer from storage site to site of erythropoiesis. Myelin protects the neuron as covering layer which is formed and maintained by Cu. It is also essential for the development of eyes, hair and skin pigment i.e., melanin (Gaetke et al. 2014). Copper is a part of cytochrome C oxidase, which catalyzes the reduction of oxygen to water, the crucial step in cellular respiration, and is a part of copper, zinc-superoxide dismutase (Cu, Zn-SOD). Nonspecific Cu++-binding to thiol enzymes transform the catalytic activities of cytochrome P450 monooxygenase, and Cu++ could both oxidize and bind to some amino acid residues of the P450 monooxygenase, but not to its heme group (Letelier et al. 2009). In brain, copper present in numerous synaptic membranes, hippocampus, basal ganglia and cerebellum (Madsen and Gitlin 2007). Many enzymes present in central nervous system like ceruloplasmin, Cu/Zn superoxide dismutase, cytochrome C oxidase and dopamine β-hydroxylase are dependent on copper for normal functioning (Pal et al. 2013).

Copper is an essential micromineral in animal's diet. It is necessary for cellular breakdown and enzymatic action such as Cu-Zn superoxide dismutase (SOD1), cytochrome C oxidase, lysyl oxidase and tyrosinase that are involved in several important procedures essential for maturation and growth (Gupta 2012). Improving diet with inorganic and organic Cu source has beneficial impacts on poultry production (Das et al. 2010; Attia et al. 2011; Samanta et al. 2011). It has been recognized that as compare to inorganic copper, supplementation of organic copper at the dose rate of 8 mg/kg is essential for the development of White Pekin ducks up to first 56 days of age. The supplementation of Cu enhanced the plasma copper cholesterol level and reduced the

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level of zinc. It has been stated that by organic copper supplementation liver Cu concentration improved along with Cu excretion and retention (Attia et al. 2012). Supplementation of copper at 10 mg/kg was effective for reproductive and productive efficacy and also improved the quality of eggs for laying hens (Attia et al. 2011). In poultry and livestock feed, $CuSO_4$ is commonly applied as feed additives (Wen et al. 2019). Supplementation of copper at 250 ppm has been certified as growth promoter (Gupta 2012).

Causes of Copper Toxicity

While excessive copper in diet is linked with several detrimental effects on poultry. Supplementation of Cu in excess amount reduced the feed intake, growth and feed conversion ratio in birds (Hashem et al. 2021). Numerous studies have verified the harmful effects of copper on birds (Yigit et al. 2012; Hefnawy and El-khaiat 2015; Huang et al. 2021). Moreover, by adding 325 ppm in poultry feed leads to initiate the muscle atrophy and growth retardation (Cinar et al. 2014). The excessive consumption of copper through feed leads to morphological modifications in the soft interior organs of the body (Attia et al. 2011). Previously, it has been described that in copper intoxicated bird's variation in liver tissue signified by necrotic and hyperplastic biliary epithelium with several deteriorative and necrotic alteration is seen at the 3rd week. Additionally, at 6th week necrosis of bile duct epithelia, cholestasis, fibroblast proliferation and lymphocytic portal aggregation occur. Long term exposure of Cu also leads to hemolytic anemia and disturb the central nervous system (CNS) (Hashem et al. 2021). Exposure to extreme level of Cu may result in oxidative distress in birds (Min et al. 2010; Yang et al. 2018), reduced the action of Cu-Zn superoxide dismutase (SOD1) and glutathione peroxidase (GSH-Px) along with increase in the contents of malondialdehyde (MDA) in duckling (Zhao et al. 2008). One of the best-known consequences of excess Cu is peroxidative damage to membrane lipids. Lipid peroxidation occurs by the reaction of lipid radicals and oxygen to form peroxy radicals. Lipid peroxy radicals may damage cells by changing the cell membrane's fluidity and permeability or by directly attacking DNA and other intracellular molecules such as proteins. In the mitochondria lipid peroxidation occurs along with in the inner membrane (cristae) Cu-damaged liver cells. Cu also leads to peroxidation in the membranes of hepatocyte lysosomes (Gaetke and Chow 2003).

Source of Copper Toxicity

Main source of copper toxicity is the accidently consumption of water supplies which contain high concentration of copper. Water may be adulterated by industrial waste or farm operation that run off into nearby public wells or reservoirs. Water running through the copper pipes may absorb copper particles and become pollutant with high copper, specifically if the pipes are corroded. Copper may be found in several types of food, drinking water and also in air. The renowned amount of copper is absorbed each day by drinking, eating and breathing. Copper level in air is typically low that's why copper exposure by breathing is minor. While people that live near smelters that process copper ore into metal, do experience this type of exposure. Occupational exposure to copper usually occurs. In the working environment copper infection may lead to flu like condition which is well known as metal fever (Royer and Sharman 2022).

Mechanism of Copper Toxicity

It is a microelement which is essential for normal functioning of body but when it accumulate in body at higher concentration, induced toxicity in aquatic life (Zeeshan et al. 2016). It is found in bounded form with protein but some time it released and in free form it catalyzed to form the reactive hydroxyl radicals. These radicals are reactive in nature, which leads to cellular toxicity. It produced oxidative stress by accumulating chronically or excessive exposure. Cu deficiency also produce oxidative stress (Gaetke and Chow 2003). When copper accumulation occurred in body, it leads to oxidative tissue damage through free radical formation (Kadiiska and Mason 2002). When Cu is found in the supply in excessive quantity than the cells demand, it excites the formation of reactive oxygen species and the abrupt deterioration of protein, fat and DNA. Several tools have been recommended to validate the Cu induced toxicity. Free Cu ions behave as an effective catalyst for the creation of free radicals. In redox reaction cupric and cuprous copper ion may execute as a vital component, i.e., cupric ion (Cu^{2+}) may be condensed to cuprous (Cu^{+}) in the presence of superoxide (O_2^{-}) that may catalyze the generation of reactive OH^{-} radicals from hydrogen peroxide (H_2O_2) breakdown by the Haber-Weiss reaction. Hydroxyl radicals are the most effective oxidative radicals possibly exist in living system and can arouse the peroxidation of lipid leading to destruction of tissues (Hashem et al. 2021). Pathogenesis of copper toxicity depend upon disruption of Cu homeostasis, due to which Cu accumulation occurred in body. Accumulation of Cu in body leads to neurodegenerative changes and hepatic disorder. Copper induced the cellular injury which leads to oxidative damage. Cellular injury result in the disruption of lipid metabolism, altered cellular events, alpha-synuclein aggregation, gene expression, Cu induced hepatic damage, initiation of acidic sphingomyelinase and release of ceramide and interaction of protein and copper in the nervous system (Gaetke et al. 2014).

Effects of Copper Intoxication

Accumulation of copper in excessive quantity leads to toxic effects such as degeneration of basal ganglia, hemolytic anemia and liver cirrhosis (Hefnawy and El-khaiat 2015).

Copper is microelement which is essential but can be harmful if its quantity increased or decreased. When copper ingested in abundant quantity, it effects adversely to the gut microflora. Liver toxicity occurred in chicken fed with CuSO4 at 1000 mg/Kg feed (Persia et al. 2003).

When rat fed with copper at 0.2 and 1.0 mg/kg BW, the AST, ALP and ALT increased significantly which showed the liver damage. Result showed that the early exposure of copper had effects on liver damage and gut microflora (Dai et al. 2020). When there is impaired copper excretion or accumulation of copper in body due to excessive intake, it leads to oxidative tissue damage through free radical formation (Kadiiska and Mason 2002). When oxidative damage occur, it causes many anomalies like Wilson’s disease (Kido et al. 2017) and Indian childhood cirrhosis (Nayak and Chitale 2013).

In a study, Male Wistar rats were injected intraperitoneally with copper lactate at 0.15 mg Cu/100 g BW daily for 90 days. Serum acetylcholinesterase (ACHE) activity was decreased significantly. The spatial memory and decreased neuromuscular incoordination was also observed as compared to control rates. Copper contents in hippocampus and in liver increased significantly to 73% and 99.1% respectively while zinc content in liver reduced to 40.7%. The hippocampus zinc content increased by 77.1% with concomitant increase in level of zinc and copper in urine and serum in those rates which were intoxicated with copper as compared to control group (Pal et al. 2013).

Copper and Oxidative Damage

Copper induced the oxidative stress through ROS production. Intoxication of copper at concentrations of 0.06 mg & 0.1 mg/L for 24 hour or 48 hour which is sub lethal concentration, showed the significant production of intracellular reactive oxygen species (ROS) in Hydra (Zeeshan et al. 2016). Copper intoxication caused the redox activity through production of reactive oxygen species (ROS) (Valko et al. 2005). It generates the hydroxyl radicals and superoxide as a result of reactive oxygen species (ROS) production via Haber–Weiss and Fenton reactions.

According to another study, body weight of the broiler birds was found decreased at 6th week which was fed with copper at the concentration of 300mg/kg feed. Feed conversion ratio (FCR) was also poor in these birds as compared to control birds feeding with normal diet. Erythrocyte superoxide dismutase, alanine aminotransferase, Plasma aspartate aminotransferase, iron, alkaline phosphatase activities, copper concentrations, and malondialdehyde were increased in copper intoxicated birds while in plasma vitamin A and C concentrations were decreased, in copper intoxicated bids as compared to control birds (Cinar et al. 2014).

Copper is also toxic to aquatic animals, depends upon its quantity/concentration (Vieira et al. 2009). Accumulation of copper in body leads to ROS production resulted in oxidative damage by oxidizing protein and lipid molecules (Wang et al. 2012). When the quantity of copper increased in aquatic medium, it resulted in the copper accumulation in hydrobionts. Lipid peroxidation occurred through ROS production in copper intoxication (Barata et al. 2005), initiation of apoptosis (Krumschnabel et al. 2005), DNA damage (Bopp et al. 2008) and agitation of embryonic development (Kong et al. 2013). Numerous effects of copper excess in human and animals have been shown in Fig. 1.

Effects of Copper Deficiency

Copper deficiency also effects adversely and shows many clinical signs like anemia, pale coat, decrease the fleece quality in sheep, decrease the capillary integrity, bone fracture, poor reproductive performance, spinal cord hypomyelinization, decreased immunity and diarrhea (Tessman et al. 2001), which leads to the economic losses. In grazing animals copper deficiency ranked at 2nd most widespread deficiency among all mineral deficiencies. When animal suffered from copper deficiency, it leads to change in blood chemistry and enzyme system (Hefnawy and El-khaiat 2015). Its deficiency adversely effects the cardiovascular system by affecting the lysyl oxidase, ceruloplasmin and Cu superoxide dismutase which are essential for the strength and integrity of blood vessels (Al-Bayati et al. 2015). It leads to fatty liver disease, if copper intake is improper (Antonucci et al. 2017). Copper deficiency may also lead to the peripheral neuropathy (Coyle 2016) and anemia (Nakagawaa et al. 2014). Numerous effects of copper deficiency on human and animals have been shown in Fig. 2.

Amelioration of Toxic Effect of Copper

The toxic effects of copper are ameliorated by Vitamin E and ascorbic acid by reversing the oxidative damage. Zinc also ameliorates the toxic effects of Cu by reversing the free radical formation after binding on that site where the Cu bound and produced toxicity. Alpha-lipoic acid, polyphenols and beta-carotene also ameliorated the oxidative stress induced by the Cu (Gaetke and Chow 2003).

In a study, liver toxicity occurred in chicken fed with CuSO4 at 1000 mg/Kg feed. Supplementation of Zn at 1 g/kg, cysteine at 5 g/ kg and of ascorbic acid 1 g/kg feed, ameliorated the toxic effects of Cu toxicity by reverting the accumulation of Cu in liver. Supplemental $ZnSO_4$ also decreased the food intake, weight gain and reduced the Cu concentration in liver (Persia et al. 2003). Copper intoxication at 300 mg/Kg feed caused the oxidative damage while supplementation of vitamin E and vitamin C at 250 mg/kg and 250 mg/kg feed respectively ameliorated the toxic effects of copper in broiler (Cinar et al. 2014). Myo-inositol (MI) ameliorate the toxic effects of Cu through reverting the ROS production, protein oxidation and induction of lipid peroxidation in fish muscle. Molybdenum also have the capability to decline the copper absorption from the intestine and stored in the liver by enhancing the copper excretion. The ratio of copper to molybdenum in total diet of sheep must be 6:1 respectively (Jiang et al. 2015).

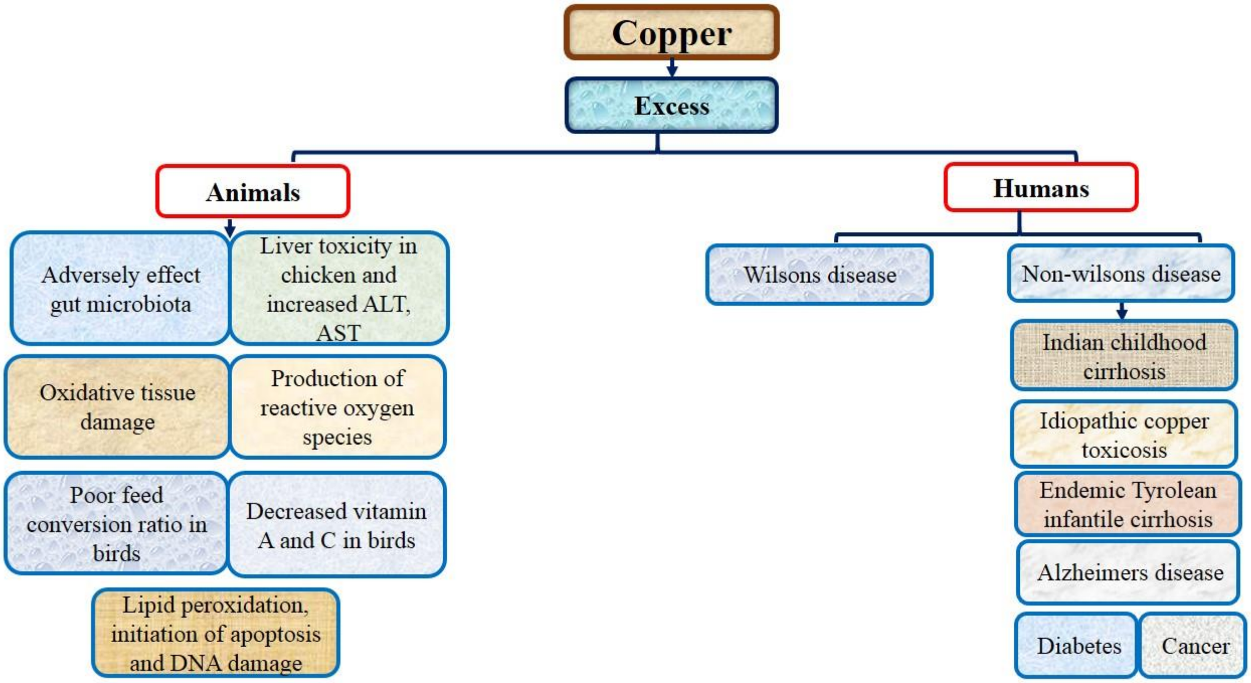
Copper
Excess
Animals
Humans
Adversely effect gut microbiota
Liver toxicity in chicken and increased ALT, AST
Oxidative tissue damage
Production of reactive oxygen species
Poor feed conversion ratio in birds
Decreased vitamin A and C in birds
Lipid peroxidation, initiation of apoptosis and DNA damage
Wilsons disease
Non-wilsons disease
Indian childhood cirrhosis
Idiopathic copper toxicosis
Endemic Tyrolean infantile cirrhosis
Alzheimers disease
Diabetes
Cancer

Fig. 1: Effects of copper excess on human and animals.

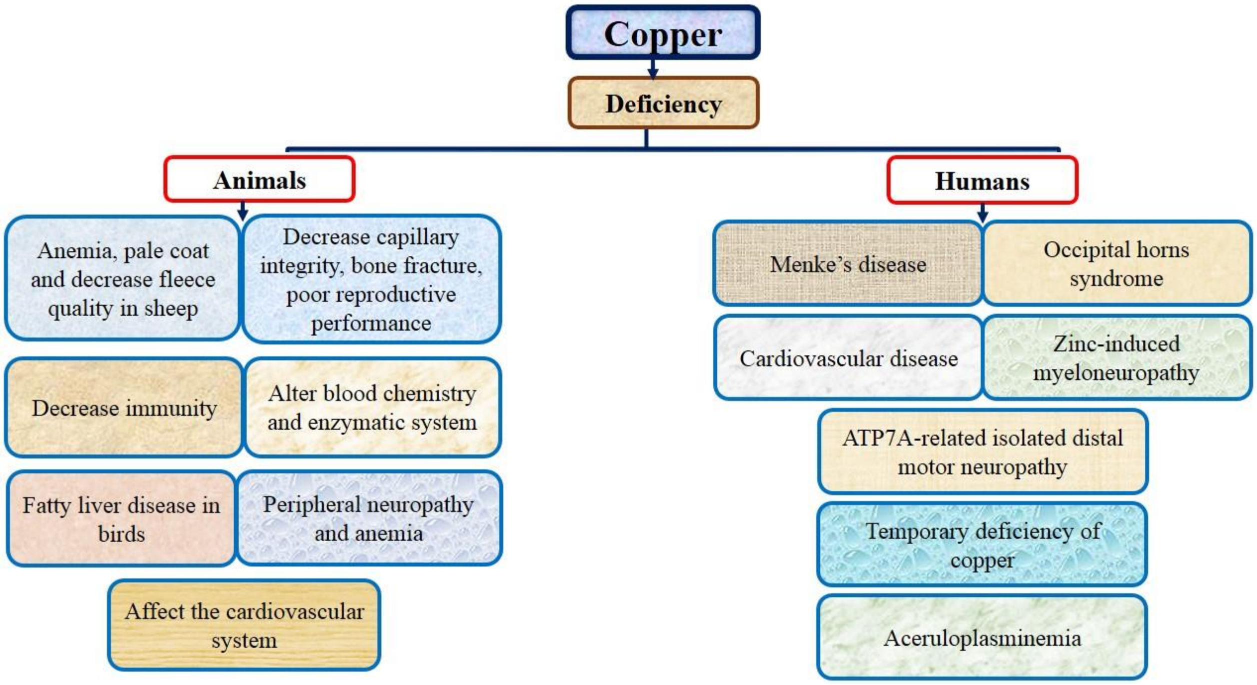
Copper
Deficiency
Animals
Humans
Anemia, pale coat and decrease fleece quality in sheep
Decrease capillary integrity, bone fracture, poor reproductive performance
Decrease immunity
Alter blood chemistry and enzymatic system
Fatty liver disease in birds
Peripheral neuropathy and anemia
Affect the cardiovascular system
Menke's disease
Occipital horns syndrome
Cardiovascular disease
Zinc-induced myeloneuropathy
ATP7A-related isolated distal motor neuropathy
Temporary deficiency of copper
Aceruloplasminemia

Fig. 2: Effects of copper deficiency on human and animals.

The consequences of Cu toxicity may be reduced by antioxidant protection system i.e., glutathione, catalase, dismutase, superoxide and vitamin C and E (Cinar et al. 2010). The dietary administration of several antioxidants i.e., selenium and vitamin C and E were effectively practiced, which mitigated the oxidative distress in animal products (Gouda et al. 2020; Amer et al. 2021; Ibrahim et al. 2021). Vitamin E is an antioxidant which protects the intracellular structure of animals and humans. It works by alleviating the harmful impacts of reactive oxygen species (ROS) and free radicals which stimulate the destruction of sulfhydryl groups and phospholipids, and leads to the altered cell membrane structure. Similarly, vitamin C is significantly critical water-soluble antioxidant, as it defends biofilms from peroxidation of lipid by eradicating the peroxyl radicals in the aqueous phase prior to the deterioration process starts (Leskovec et al. 2018). It also acts to reload the declined vitamin E. The lipophilic radicals form in the membranes may be eradicated directly by vitamin C, while it declines the quantity of tocopheroxyl radicals that are observed in the membrane during the conversion of the lipophilic to aqueous phase. Several studies exposed good performance by the supplementation of vitamin C and E in poultry (Cinar et al. 2014; Zhu et al. 2019; Gouda et al. 2020; Amer et al. 2021) or fish diet (Ibrahim et al. 2020; Ibrahim et al. 2021; Azeez and Braimah 2020; Ahmed et al. 2021). Currently, several distinct information's have stated that vitamin E and C may defend from lethal impurities of xenobiotics and minerals due to their vital role as an antioxidant (Ajuwon and Idowu 2010). Both vitamins may work synergistically to prevent the harmful effects of copper toxicity (Cinar et al. 2014). Previously it was described that concurrent impacts of vitamin C and E may alleviate the DNA and histological alterations in the liver of birds intoxicated with CuSO4. Vitamin C and E alleviate the oxidative stress, Leukogram, erythrogram alterations and histopathological alteration in kidney induced by CuSO4 toxicity in birds (Hashem et al. 2021).

As copper is able to catalyze the development of reactive oxygen species, while the nutrients having the antioxidant abilities may protect against copper induced oxidative injury. Possible mechanism for antioxidant action of nutrients comprises of; constrain the formation of mitochondrial superoxide, scavenge or chelate the reactive oxygen species, or withdraw the transitional metal ions from the site of reactive oxygen species formation, declining the hydroperoxides formation, and restoration of impaired molecules. Significant enzymatic antioxidant systems in the body comprise of Mn-SOD, CU, Zn-SOD, GSH-Px and catalases, while non-enzymatic antioxidant system comprises of vitamin C, GSH and beta-carotene (Gaetke and Chow 2003).

Conclusion

Copper is a vital nutrient and a redox-active transition metal which can stimulate oxidative injury. High level of Cu may lead to enhanced oxidative destruction of protein, lipid and DNA and subsidize to neurodegenerative syndromes. Alternatively, copper is necessary for optimum antioxidant protection, and copper deficiency declines the body capability to deal with oxidative distress. Several nutrients can intermingle with copper and change its toxicity. Available evidences indicate that vitamin C and E, ascorbic acid, zinc, alpha-lipoic acid, beta-carotene, molybdenum and polyphenol primarily protects against the copper induced oxidative injury.

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24

Anti-diabetic Properties of *Chenopodium quinoa* on Human Health; An Update and Future Prospects

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INTRODUCTION

There are many disorders which acutely affect human being and are prevalent across the world. Among these, diabetes mellitus occurs abundantly in the developing countries. Diabetes is a chronic condition that occurs when there are raised levels of glucose in the blood because the body cannot produce any or enough of the hormone insulin or use insulin effectively. According to International Diabetes Federation (IDF), in Pakistan every 4th person is affected with diabetes. It is generally classified as type-1, type-2, and gestational diabetes. The occurrence of type-2 diabetes is much more than the type-1 and gestational diabetes (Atkinson et al. 2014). Due to numerous side effects of allopathic treatment, scientists are now moving toward botanicals showing good efficacy. Qunioa (*Chenopodium quinoa* wild) is crop belongs to the Amaranthaceae family; sub family Chenopodioideae and considered as pseudocereal. Its edible seeds are gluten free containing various minerals (P, K, and Mg) and vitamins (Vitamin B1, B2, B3, B6, C and E) which are effective against numerous diseases. Raw uncooked quinoa consists of 13% water, 64% carbohydrates, 14% proteins, and 6% fat. The saponins (glycosides) protection on seed make it unpalatable but protect it against many biotic factors including insect and birds which harshly affect the potential yield (James 2009). Qunioa can be effectively used against many diseases such as cancer, cardiovascular diseases, and diabetes mellitus. The anti-diabetic effects of quinoa are due to the presence of various chemicals which lowered the blood glucose level. It also releases many phytochemicals which help in making an efficient diet for diabetic patients (Graf et al. 2014). This chapter broadly discusses the anti-diabetic impact of quinoa on human health.

Diabetes Mellitus

Diabetes is an autoimmune disease, in which β-cells of the pancreatic islet are attacked by the host immune system, leading to impaired growth and function. Regarding the possible causes of Type-1 Diabetes Mellitus (T1D), in 70-90% of diabetic cases, autoimmunity is the prominent cause of damaging β-cells (Atkinson et al. 2014). Diabetes is mainly divided into two types including type-1 and type-2 diabetes mellitus (T2D) in which T2D (not dependent on insulin) depends on the existence of autoreactive antibodies leading to the damage of β-cells. T2D is considered as an epidemic in multiple countries, especially in developing countries, and one of the major causes of death. Nowadays it has become a major global health concern (Bommer et al. 2018). According to hygiene hypothesis, good hygiene may contribute to the occurrence of diabetes. A precise estimation done by International Diabetes Federation (IDF) in 2021, 536.6 million individuals are suffering from diabetes and this value is expected to extend by 46%, reaching 783.2 million by the year 2045 (Sun Hong et al. 2021). In diabetes, body is unable to differentiate between the invading pathogen and body own cells leading to the attack on the body cells by immune system (Arif et al. 2004). A chronic helminth infection may decrease the occurrence of diabetes due to immune shift indicating an alternative way to control diabetes mellitus (Klement et al. 2008; Hubner et al. 2012).

Mechanism of T1D Development

Typically, after taking the meal, along with insulin production, glucose is also produced in the body which causes an increase in the biotransformation of sugar molecules that are deposited in muscles and fat tissues. Without dependence on insulin, glucose is provided to the brain by the liver during fasting conditions. On the other side, insulin inhibits glucagon secretion in response to which serum fatty acids are produced in low concentrations resulting in decreased production of glucose by the liver (Kangralkar et al. 2010).

Blood hyperglycemia results due to insulin resistance or low assembling of insulin in the blood that hampers the process of glucose uptake in tissue. Because of impaired assembling

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of glucose in the blood, the process of gluconeogenesis and glycogenesis initiates which resulted in fat breakdown and it directed toward diabetic ketoacidosis. Hyperglycemic coma is due to the rising level of glucose in the blood. Less assembling of insulin in the blood may result in the destruction of pancreatic β-cells due to different causes, named as T1DM (Type-1 Diabetes Mellitus). Principal factors which cause the destruction of β-cells are environment, autoimmunity, and a genetic disorder. Because of insulin resistance, there is impeded regulation of insulin which causes another condition called Type-2 Diabetes Mellitus (T2D) (Asmat et al. 2016). Mechanism of glucose management in Liver and pancreas Asis shown in Fig. 1.

Diabetic victims are always at threat with several hurdles ranging from macrovascular to microvascular glitches. Stroke and coronary heart disease are embodied in macrovascular barriers while nephropathy, retinopathy, and neuropathy are included in microvascular complications. The hazard of macrovascular deterioration is more in aged people than in microvascular complications. It is assessed that by 2035, 600 million individuals are to be treated with diabetes and this autoimmune disease is rising extensively around the globe (Berbudi et al. 2016).

Quinoa (*Chenopodium quinoa* wild)

Quinoa, a member of Amaranthaceae family, is an indigenous crop that shows resemblance to cereal crops and primitively grown in the Andean region of South America, including Ecuador, Chile, Peru, Bolivia, and Colombia. From 3000–4000 years, this magnificent crop has been cultivated for livestock feed and for the consumption of human beings (Vega-Galvez et al. 2010). It was considered the principal meal of the Inca civilization. The dominantly spoken language of the Incas was Quechua. Therefore, citizens of the Incas called quinoa; chisaya mama "mother of all the seeds,", because quinoa food was represented together with potato and maize and considered as a chief dietic element. Therefore, it is known as "Inca gold" because of its marvelous healing and beneficial properties. While, in current years quinoa is introduced in different continents and countries of the world including Japan, North America, Europe, China and Australia. Due to present situations, quinoa is restricted to South America only. Hence, quinoa was re-introduced for its nutritional and agronomic attributes (Hussain et al. 2021). It is herbaceous annual plant grown specifically for edible seeds. In comparison with other cereal crops, it contains a high amount of dietary fiber, protein, dietary minerals, vitamin B, and a low level of glycemic index that reduces the gluten concentration making it a beneficial crop for diabetic patients (González Martín et al. 2014). It is considered as a pseudo-cereal crop and has similar characteristics to the amaranth and spinach families (Fuentes et al. 2009). The genus of quinoa is Chenopodium, and around 250 species have been identified all over the world. United Nations declared the international year of quinoa in 2013 to highlight its tremendous potential (Tang et al. 2015b). The acclimatized species of genus *Chenopodium* are categorized into two parts based on crossing relationships, pericarp and perianth morphology (Adolf et al. 2012).

Morphological Characteristics

Quinoa is monoecious annual plant, with an erect stem and alternate leaves colored due to pigments like betacyanin. In India, several varieties are sown, and reliable growth is expressed by plants up to a height of 1.5m, habitually, with large leaf sizes and a large number of branches. The lower and upper leaves have rhomboids and lanceolate shapes, respectively (Gomez and Pando 2015). Petiole and lamina are the components of leaves; petioles are grooved, long and tenuous. The length of the petiole fluctuates sometimes within the same plant and depends on the variety. The leaf blade of a plant shows polymorphism within the same plant; for instance, it can be triangular, wavy, flat, or diamond-shaped (Christensen et al. 2007). The quinoa plant is associated with intense branching and deep root system, which plays a significant role in plant stability on the ground and allows the plant to suffer under drought conditions. The stem of the quinoa plant is woody in nature, erected and ramified.

The inflorescence of quinoa is commonly called panicle, comprising of secondary and tertiary branches along with pedicels holding glomeruli. The length and the diameter of the panicle ranges from 30 to 80 cm and 5 to 30 cm respectively and variability in both depends upon types of genotypes and fertility status of the soil. The number of glomeruli per panicle varies between 80 to 120, while the range of seeds per panicle is up to 3000 (Mujica et al. 2001). Quinoa fruits have ellipsoidal, spherical, and conical shapes and the pericarp of fruit varies in color from yellow to greyish with magenta. Due to the small size of fruits, the weight falls in the range of 1.9 to 4.3g per 1000 seed weight (Kozioł 1992). The embryo, perisperm and episperm are three vital components of seeds (Prego et al. 1998).

Nutritional Profile

Quinoa grains are an excellent source of indigestible compounds with biological and antioxidant properties (Pellegrini et al. 2018). The nutritional profile of quinoa varies significantly among genotypes and has been categorized into two group; the group A is polyunsaturated fatty acids and phytochemicals present in high amount, and group B consists of long-chain fatty acids and linolenic acid in high concentrations (Nowak et al. 2016; Chen et al. 2019). Quinoa grains have high nutritional value consisting of protein (10–18%), starch (32–60%), fats (4.4–8.8%), ash content (2.4–3.7%) with P and K being the primary ash sources and a fiber

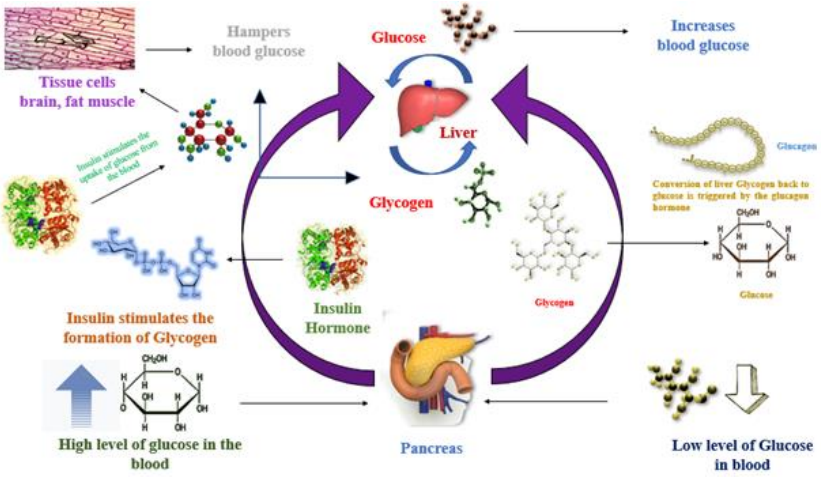

Fig. 1: Mechanism of glucose management in Liver and pancreas.

Table 1: The quinoa's general nutritional profile and comparison to the nutritional profiles of other foods (University of Sydney 2014; USDA 2015).

Food	Carbohydrates(g)	Fiber (g)	Lipids (g)	Proteins (g)	Energy (kcals)	Glycemic index (GI)
Rice	28.59	0.3	0.21	2.38	130	75-89
Corn	20.98	2.4	1.5	3.41	96	60
Wheat	25.12	4.23	0.66	3.6	113	48
Quinoa	21.3	2.8	1.92	4.4	120	35-53

(1.1–13.4%) (Navruz-Varli and Sanlier 2016; Romano et al. 2020). Quinoa grain is a raw material with a high carbohydrate content made up of starch and low sugar content. Due to high nutritional value of quinoa, it is a complete food, mainly due to good quality protein and minerals such as Mg Ca, Fe, Mn, P, Zn, and Cu (Vega-Galvez et al. 2010). It can be used as "functional food" and diminishes the risks related to many diseases. The presence of minerals, vitamins, fiber, antioxidants, fatty acids and plant hormones in quinoa dominates its functional properties that subsidize the nutritional aspects of humans (Repo-Carrasco-Valencia et al. 2011). Comparison of nutritional composition of cooked quinoa to other common cereals is summarized in Table 1.

Biochemical Configuration

Proteins

As proved from previous studies, quinoa is rich in protein and the dietic proteins originate from its grains provide all types of cardinal amino acids. The protein content in quinoa ranges from 12 to 23% (Comino et al. 2013; Fotschki et al. 2020). It is considered as a perfect food as it contains all essential amino acids i.e., histidine, tryptophan, leucine, isoleucine, lysine, phenylalanine, methionine, tyrosine, threonine, and valine (Maradini-Filho 2017). The percentage of globulins and albumins in the grains of quinoa are about 37% and 35%, respectively (Miranda et al. 2012). On quality basis, quinoa proteins are comparable to the protein (casein) present in the milk. An 11S-type protein globulin with a quaternary structure is one of the proteins found in quinoa consisting of a variable content of asparagine, aspartic acid, serine, glycine, leucine, arginine, and glutamine–glutamic acid. By comparing with the total composition of quinoa seed protein, it has approximately low sulfur-containing amino acids. Cysteine, arginine and histidine are the amino acid comprising another protein known as 2S-type albumin (Filho et al. 2017).

Vitamins

Folic acid and vitamins B and E are assembled in the embryo of quinoa. It is a much more reliable source of these vitamins (Basantes-Morales et al. 2019). In comparison to other cereals, the grains of quinoa consist of a large amount of riboflavin, vitamin C, thiamine, and folic acid. After the consumption of 100g of quinoa, an adult can easily meet his dietary necessities. 80% of the dietary requirements of children and 40% of the cellulase, protease, and hemi cellulase requirements are fulfilled after consuming 100g of quinoa grains (Mohyuddin et al. 2019). Beta-carotene, which is a precursor of vitamin A, weighs 0.39 mg per 100g of quinoa; tocopherols/vitamin E range from 3.7 to 6.0 mg per 100g, riboflavin/vitamin B2 weighs 0.39 mg; niacin/vitamin B3 weighs 1.06 mg per 100g, pyridoxine/vitamin B6 weighs 0.20 mg per 100g; pantothenic acid/vitamin B5 is 0.61 mg per 100 g, folic acid/vitamin B9 23.5 to 78.1 mg per 100 g, ascorbic acid/vitamin C 4.0 to 16.4 mg per 100 g (Tang et al. 2015a). Amounts of different Vitamins present in per 100g of Raw and polished quinoa seeds are shown in Table 2.

Table 2: The content of certain vitamins in quinoa seeds (mg/100g dry basis) (Ruales and Nair 1993).

Vitamins	Raw whole Quinoa	Polished and washed quinoa
Riboflavin	0.2	0.2
Vitamin C	16.4	19.2
Vitamin A	0.2	0.2
Folic Acid	78.1	66.3
Tocopherols		
γ-tocopherols	5.3	4.9
α-tocotrienols	ND	ND
δ-tocopherols	0.3	0.3
β-tocotrienols	0.3	0.3
α-tocopherols	2.6	2.4
β-tocopherols	0.2	0.2
γ-tocotrienols	0.01	0.01

Table 3: Mineral composition of Quinoa seed (Nowak et al. 2016).

Minerals	Amount
P (%)	0.47
Ca (%)	0.19
K (%)	0.87
Mg (%)	0.26
Mn (ppm)	128
Fe (ppm)	205
Zn (ppm)	50
Cu (ppm)	67
Na (ppm)	115

Table 4: Carbohydrate content in grains of quinoa (Jan et al. 2019)

Quinoa Genotypes	Carbohydrates (%)
INIA-415 Pasankalla	67
Ccoito	68.1
03-21-1181	69.8
Witulla	69.5
Salcedo INIA	70
03-21-0093	Not detectable
Huaripongo	67.8
Commercial 2	59.4
Commercial 1	63.6
Roja de Copotaque	70.8

Minerals

Quinoa grain contain high concentration of Fe, Cu, Zn, Mg and Ca as compared to other cereals, including wheat, oats, triticale, rice and barley (Filho et al. 2017). The percentages of Ca, Fe and Zn in quinoa grains are 39.43%, 49.04% and 20.25%, respectively (Darwish et al., 2020). Cu (2 to 51 mg/kg), Zn (28 to 48 mg/kg), P (1400 to 5300 mg/kg), Mg (260 to 5020 mg/kg), K (75 to 12000 mg/kg), Ca (275 to 1487 mg/kg) and Fe (14 to 168 mg/kg) are the micronutrient found in quinoa grains. Therefore, for a balanced human diet, micronutrients in quinoa are in ample quantities (Vega-Galvez et al. 2010). Amounts of minerals present in quinoa seeds are enlisted Table 3.

Carbohydrate

The amount of carbohydrates is directly linked with many metabolic processes like protein glycosylation and plays a pivotal role in the nutritional aspects. Seeds of quinoa exhibit some beneficial characteristics like lowering the free fatty acids resulted in reducing hypoglycemic effects. The concentration of gluten is negligible in quinoa grain as compared to wheat, oat etc. that make it a prominent cereal (Hussain et al. 2021). As reported by Jancurová et al. (2009), a remarkable number of carbohydrates in the range of 67–74% of total dry matter are present in quinoa, and nearly 11% of amylose content is present. Concurrently, some distinct carbohydrates are also cataloged, including crude fiber (2.5–3.9%), pentosans (2.9–3.6%), monosaccharides (2%), and disaccharides (2.3%) (Valencia-Chamorro et al. 2003). The starch present in quinoa is described by its high digestibility and solubility. Up to 58.1–64.2% of the dry matter of quinoa is composed of starch, and the concentration of amylose is also low (around 10%), although the concentration fluctuates between 3% to 20%. Around 90% of quinoa starch is made by amylopectin. Quinoa's gelling point is adequately lower under cold temperature storage. Its seeds are characterized by their creamy nature and smooth texture (Vega-Galvez et al. 2010). In comparison, sucrose is present in a substantial quantity (Schoenlechner, 2017). The carbohydrate composition of different genotypes is enlisted in Table 4.

Fiber

Quinoa is considered as a chief source of fiber. Fiber content is not markedly affected by the abrading and washing of quinoa with the purpose of eliminating the saponins (Ranhotra et al. 1993). In quinoa the content of dietary fiber is equivalent to legume grains and cereals, but as compared to buckwheat, low dietary content is present in quinoa (Alvarez-Jubete et al. 2009). As reported by Lamothe et al. (2015), the total dietary fiber present in quinoa is 10%. Fiber is a fraction of carbohydrates that are resistant to absorption in the small intestine and enzymatic digestion, but in the large intestine, it normally undergoes partial or full fermentation. Fiber can improve the intestinal microbiota, low cholesterol, reduce the risk of gastrointestinal infections and inflammation (Brownawell et al. 2012; De Carvalho et al. 2014).

Lipids

Quinoa contains 5.3%–14.5% lipids and the lipid content of quinoa is distinguished by a high degree of unsaturation with the range of 70% to 89.4% (Gordillo-Bastidas et al. 2016). Lipid is usually located in embryo of quinoa and the range of fat content is also quite high (5–10%) (Prego et al. 1998). About 25% of the total lipids are characterized by polar lipids, mainly phospholipids such as choline and lysophosphatidic ethanolamine (Benito et al. 2018). The stability of the oil is particularly dependent on natural antioxidants that exist in high quantities including α -tocopherols (69-75 mg/100 g of oil) and γ-tocopherols (76-

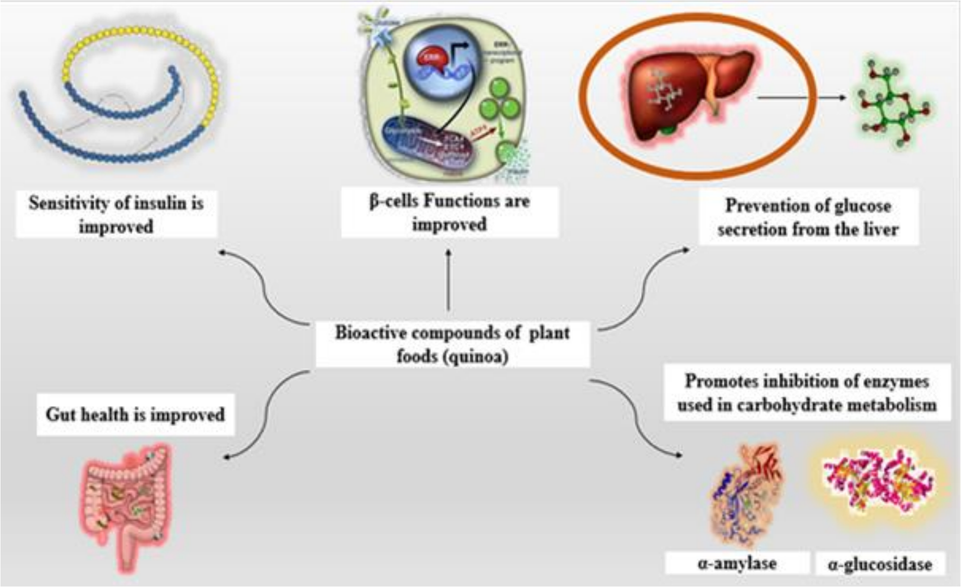
Sensitivity of insulin is improved
β-cells Functions are improved
Prevention of glucose secretion from the liver
Bioactive compounds of plant foods (quinoa)
Gut health is improved
Promotes inhibition of enzymes used in carbohydrate metabolism
α-amylase
α-glucosidase

Fig. 2: Functions of bioactive compounds found in *Chenopodium Quinoa.*

Table 5: Functions and biological activities of chemical contents lie in *Chenopodium quinoa.*

Compound	Bioactivity	Plant parts	References
Chlorogenic acid	Anti-diabetic	Seeds & leaves	Tang et al. 2016
20-Hydroxyecdysone S	Anti-diabetic	Seeds	Foucault et al. 2012
Betaine, choline	Anti-diabetic	Seeds	(Olthof and Verhoef 2005)
β-Sitosterol	Anti-diabetic	Seeds	Ahamed et al. 1998

93 mg/100 g of oil) (Kozioł 1992). A contemporary study indicates that 89.4% unsaturated fatty acids are found in quinoa seed oil containing 54.2% to 58.3% polyunsaturated fatty acids (Tang et al. 2015a). Quinoa seed is considered as the primary source of several essential fatty acids, including omega-6 and omega-3. Different phytosterols are also present in quinoa, mainly squalene (Graf et al. 2015).

Anti-diabetic Nature

Pharmacological Activity of Quinoa

Quinoa contains a variety of bioactive compounds, particularly in its seeds, stems, leaves, and roots. These bioactive compounds have many beneficial biological consequences including antioxidant, anti-inflammatory, anti-diabetic, anti-fungal, anti-microbial and anti-cancer effects (Table 5) (Shah et al. 2022).

Antidiabetic Consequences

Quinoa contains higher levels of bioactive peptides, protein, fiber, tocopherols, and phenolic acids, which are well known for their antidiabetic attributes (Cisneros-Yupanqui et al. 2020). In diabetic patients, HbA1c and BMI levels can be controlled after consumption of quinoa. Furthermore, quinoa aids in the maintenance of blood glucose levels and the improvement of health in people with prediabetes (Abellan et al. 2017). Graf et al. (2014) studied the impact of some compounds such as phytoecdysteroids which are leached by the seeds of quinoa. For the objective, with leaching efficiency being optimized, an 80°C temperature, a 70% ethanol concentration, a period of 4 hours, and a 5 ml/g solvent ratio were used. Quinoa leachate with the compounds and markedly lowered the blood glucose level in mice which were obese and hyperglycemic. In another study by Li et al. (2018), glucose and fat levels were assessed after consumption of quinoa-enriched bread in patients with cardiovascular disorders, and it was unveiled that the glucose levels in the control group were low. Hanan et al. (2019) examined the anti-diabetic and antioxidant activity of quinoa seed powder in diabetic rats. Rats blood and glucose levels were minimized after consumption of quinoa in different concentrations. Furthermore, rats were fed with several concentrations of quinoa seed powder which resulted in the reduced level of thyroid hormones. There are several other plants from which phenolic compounds exhibit in vitro α-glucosidase and α-amylase inhibitory consequences (Hemalatha et al. 2016). Another research which was performed in vitro demonstrated the quinoa polysaccharides inhibitory effects on α -glucosidase and α-amylase (Tan et al. 2020). Peptides located in quinoa might assist in controlling the blood sugar levels as the α-amylase and α-glucosidase dipeptidyl-peptidase-4 (DPP-IV) are influenced by inhibitory effects of quinoa peptides (Nongonierma et al. 2015; Vilcacundo et al. 2017; Guo et al. 2020; Mudgil et al. 2020). For the remedy of diabetes mellitus type-2, DPP-IV inhibitors are efficiently used as they assist to reduce the fasting hyperglycemia by protracting insulin secretion (Green et al. 2006; Kasina and Baradhi 2019). Moreover, it is stated that hypoglycemic potential is also present in the saponins (Marrelli et al. 2016; Ren et al. 2017; Singh et al. 2017). The process of digestion and gastric emptying slow up due to high protein content of quinoa (Cisneros-Yupanqui et al. 2020). Various functions of bioactive compounds found in *Chenopodium Quinoa* are illustrated in Fig. 2.

The hypoglycemic potential of quinoa has been proved in many in-vitro studies (Al-Qabba et al. 2020; Cisneros-Yupanqui et al. 2020; Noratto et al. 2019; Ujiroghene et al. 2019; Ayyash et al. 2018; Hemalatha et al. 2016; Graf et al. 2014). According to Hemalatha et al. (2016), quinoa extract can inhibit the enzymes α-amylase and α-glucosidase. In another study, a low glycemic diet including quinoa was tested in diabetic patients and the results indicated a drop in HbA1c and increase in HDL (1.7 mg/dL) level (Pasko et al. 2010b).

Conclusion

Chenopodium quinoa attains a nutritionally rich profile, including lipids, carbohydrates, proteins, vitamins, and minerals. It is a superfood (gluten-free diet) for humans and is relatively much better than other cereals. It has an exceptional and beneficial composition, including fiber, phenolics, bioactive peptides, proteins, and tocopherols. It is very effective against certain diseases such as cardiovascular issues, celiac disease, and especially against T_2D. Diabetes is inhibited by its enzyme inhibition properties and the production of less glucose, which stabilizes the intensity of the disease. If it is consumed on daily basis, it can overcome diabetes and other diseases more efficiently than a synthetic drug.

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A Recent Examination of the Impact of Selenium Supplementation to Lactating Cattle

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INTRODUCTION

An adequate dietary intake of minerals (macro- and micronutrients) is fundamental for any cellular life on earth. Among micronutrients, selenium has been the subject of a continuous debate when it comes to human as well as animal nutrition. From toxicity to deficiency, research on selenium has come a long way since its discovery in 1817 by the Swedish chemist Jons Jacob Berzelius (Cobo-Angel et al. 2014).

Like any other animal enterprise, selenium is shown to be an indispensable element in dairy nutrition promoting the overall health status of animal viz. improving gut health, regulating immune system, balancing thyroid hormones, contributing to disease resistance and boosting reproductive performance (Séboussi et al. 2016; Arshad et al. 2021; Xiao et al. 2021). All these vital roles of selenium originate from the fact that it is incorporated in range of selenoproteins (Labunskyy et al. 2014) which are directly or indirectly involved in antioxidant defences (Sun et al. 2019). Research has established that synthesis of selenoproteins is affected by the nutritional level of selenium (Han et al. 2021). Thus, low levels of selenium in diet or, more precisely reduced selenoprotein synthesis in body ultimately affects various metabolic processes (Pedrero and Madrid 2009).

Dietary supplementation of different selenium compounds to farm animals also enhance the nutritional quality of final food product. All these valuable contributions in livestock make selenium a fascinating nutrient, and any half-measures in its supplementation would have far reaching consequences other than compromised animal health and production (Salman et al. 2013; Ianni et al. 2019).

Requirement

Ruminants exclusively depend on diets of plant origin as source of fiber along with other nutrients. These plants, via conversion of selenium salts to different organic forms (mainly selenomethionine) and their subsequent non-specific incorporation into plant proteins, introduces the selenium into human and livestock diets (Calamari et al. 2010). Most of the world soils are deficient in selenium, and different forages grown on them are unable to meet animal requirements (Filley et al. 2007; Sordillo 2013). Moreover, extensive farming strategies along with the legislature prohibiting the use of selenium-based fertilizers inflict lower selenium levels in the soil which consequently translate into lower intake by livestock, thus, necessitating external supplementation of selenium (Davy et al. 2016).

The National Research Council (2001) has set dairy cattle selenium requirement at 0.3 mg/kg feed (DM basis) irrespective of age, physiological stage of animal and selenium form (Oltramari et al. 2014). These propositions most probably intended to prevent the element deficiency in animal rather than health optimization or toxicity prevention (Sordillo 2013). National Research Council (2005) recognized 5 mg selenium/kg feed (DM basis) as maximum tolerable level for ruminants. For dairy nutrition, United States (Food and Drug Administration, 2003) and Canada (Canadian Food Inspection Agency 2015), limit external selenium supplementation to 0.3mg/kg dry matter regardless of the form used, which is roughly equivalent to 3 mg selenium per animal per day. In the European Union, cattle can be fed diets with a maximum of 0.5 mg/kg DM of total dietary selenium with 0.2 mg/kg DM organic selenium at the most (EFSA 2013). Recently, Hendriks and Laven (2020) compared selenium allowances for dairy cattle raised in New Zealand with other parts of the world; attributing to dominant pasture-based diet for better part of the year with adequate vitamin E and polyunsaturated fats. They recommended that feeding the cattle at 0.03 mg selenium/kg DM should be enough.

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In dairy, a single fixed recommendation for dietary selenium seems irrational. The generally accepted recommendation (0.3mg/kg DM) may not be sufficient for cattle at a particular stage (transition period) or when animal is under stress (heat stress) (Gong and Xiao 2021). During the pre- and postpartum period in cows, beneficial role of dietary selenium at slightly higher concentrations than recommended have already been tested in many studies (Kamada 2017; Khalili et al. 2019). Based on various factors viz. animal productivity, body vitamin E status, ingestion of polyunsaturated fatty acids, ambient temperature etc. Suttle (2010) articulated a hierarchy of selenium requirement in cattle which suggested that it is the balance between anti- and prooxidants at any stage or time which determines selenium demand of animal. Increase in body oxidant state will increase the body selenium requirements and vice versa (Hendriks and Laven 2020). A good deal of published studies also stated manifold increase in milk selenium concentration when cattle were fed with supra-nutritional quantities of selenium (Givens et al. 2004; Walker et al. 2010; Sun et al. 2021). However, farmers and legislation authorities must be attentive to the threat posed by such high levels of selenium in animal diets to avoid any environmental hazard. For this, considerable attention should be given to the form of selenium present in the feed supplement.

Sources (inorganic vs. organic)

A great deal of information has been accumulated over a span of years indicating the paramount importance of dietary form of selenium when it comes to bioactivity and metabolic fate of the element (Suttle 2010). In general, there are two selenium sources in cattle diets: (A) native feedstuffs mainly comprising roughages and grains (may include drinking water), and (B) supplemental selenium primarily via mineral premixes or salt blocks. Typically, feedstuffs of plant origin contain selenomethionine accounting for 55-65% of selenium (Whanger 2002) whereas external supplemental sources exist in either inorganic (salts of selenite and selenate) or organic form (selenium yeast or pure selenomethionine preparations) (Surai 2006; Silvestre et al. 2007) (Fig. 1).

Various studies have been made to assess the role of inorganic and organic selenium in dairy nutrition, based specifically on bioavailability, state of production and reproduction, body antioxidative status, tissue accumulation, udder health, milk transference, dam-calf transfer efficiency, toxicity risk assessment and environmental pollution. Most often, such comparative analyses showed notable positive responses with organic forms. Therefore, in recent years, utilization of organic selenium supplements in livestock nutrition has gained considerable interest, however, relatively low prices of inorganic salts still entangle the usage of organic supplements in practical farming (Weiss 2005; Sun et al. 2017).

The bio-efficiency of organic forms revolve around their selenomethionine content rather than total selenium levels (Juniper et al. 2019). Surai et al. (2017) summarized the commercial selenium products into three generations: inorganic selenium salts being the first but outdated one; selenium yeast, pure selenomethionine and Zn-selenomethionine comprising the second generation; hydroxy-selenomethionine, the third generation. Limited productivity of inorganic forms, complications in producing yeast products with constant selenomethionine levels, and instability (oxidation) of the pure selenomethionine supplements paved the way for hydroxy selenomethionine products. They also questioned eligibility of nano selenium as organic supplement along with chelated selenium products viz. glycinates, proteinates and various amino acid complexes, as these are not the methionine bound forms.

Other than application of selenium into ruminant diets via mineral premixes, there have been attempts to increase the selenium concentration of native forages using selenium-based fertilizers (Cun et al. 2015; Séboussi et al. 2016). However, due to the narrow window between selenium toxicity and necessity, there are legislative restrictions, and not all the countries allow the use of selenium fertilizers (Tremblay et al. 2015). In any case, learned decisions involving selenium supplementation in an optimal form and amount are vital in any livestock enterprise to prevent deficiency diseases along with toxicity and environmental pollution.

Absorption and Metabolism

In dairy cattle, metabolic fate of dietary selenium is complicated by the rumen physiology when compared to non-ruminants. Thus, rumen is the major determinant of the nutritional value of given selenium source, even though duodenum is the part involved with most of the absorption. Metabolic fate of ingested selenium from different dietary sources in dairy cattle is shown in Fig. 2. Young ruminants absorb dietary selenium with almost similar efficiency as non-ruminants (Freer 2007; Salles et al. 2017).

Ruminal metabolism of inorganic selenium involves the conversion of selenate (firstly reduced to selenite) and selenite to low molecular insoluble forms which are supposedly not well utilized by animal and are excreted from the body (Salles et al. 2017). Literature also showed synthesis of selenoamino acids (predominantly Se-cysteine) in rumen from selenite, which are later incorporated into microbial protein. Besides, some quantities of ingested selenate and selenite also pass the rumen unchanged and are absorbed in lower part of the gastro-intestinal tract by active and passive pathways, respectively. In a trial with mid-lactation cows, Wei et al. (2019) found selenium apparent absorption of 49.5 and 59.7% with sodium selenite and hydroxy analogue of selenomethionine, respectively. Poor absorption of inorganic selenium could be attributed to the ruminal environment where microorganisms reduced them to insoluble forms. Niwińska and Andrzejewski (2017) unveiled that coating of

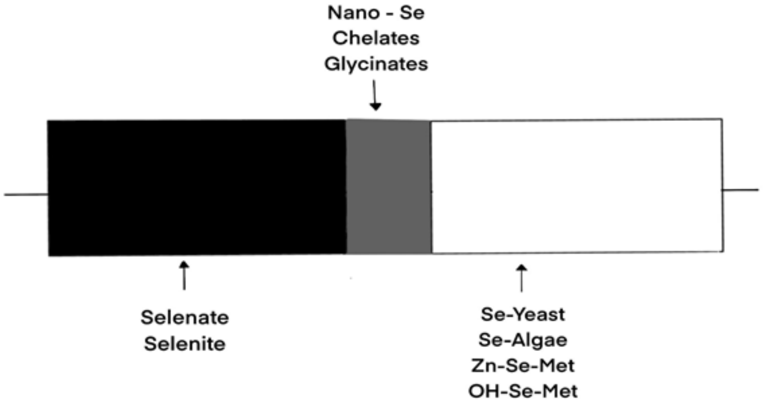

Fig. 1: Major commercialy available selenium supplement

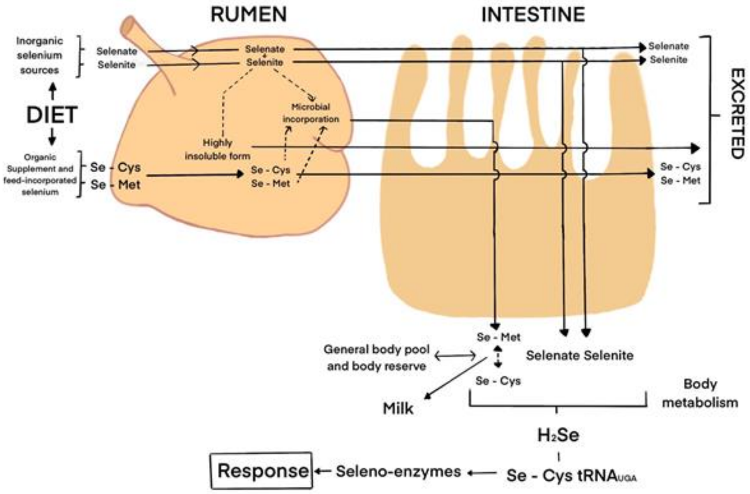

Fig. 2: Metabolic fate of ingested selenium from different diatetary sources in dairy cattle.

inorganic selenium sources with rumen insoluble compounds could increase their availability, supposedly by increasing their incorporation into microbial proteins instead of being reduced to insoluble forms.

Conversely, organic forms packed with selenoamino acids (mainly selenomethionine) encounter fewer alterations in rumen than inorganic sources. Selenomethionine and selenocysteine, upon reaching the small intestine, are absorbed following the routes similar to methionine and cysteine. A higher proportion of selenium from selenomethionine is incorporated into microbial protein than selenite (Mainville et al. 2009). Weiss (2005) stated that greater amounts of selenium as selenomethionine escape the rumen with selenium yeast feeding than selenite or selenate.

The increase uptake of selenium by ruminal microbes might be the reason behind improved bioavailability of selenium in small intestine of ruminants (Hall et al. 2012). Furthermore, in a comparison between selenium yeast and selenium-fertilized forages, Séboussi et al. (2016) reported higher selenium availability from forages and suggested higher digestibility of selenium fed with biofortified forages than selenium yeast, although cattle intake of selenium was higher in forage diets. Nonetheless, in addition to the variable digestion of forages and yeast wall, they attributed these results to longer ruminal retention time of forages and better selenium dispersion in the ration and rumen leading to better selenium accumulation into microbial proteins.

Irrespective of the form absorbed (selenite, selenate, Se-Met, Se-Cysteine), selenium must be metabolically reduced to inorganic selenide which is then used in the synthesis of Se-Cysteine-tRNA, an active centre for various selenoenzymes (Suzuki and Ogra 2002; Surai 2006). Direct insertion of absorbed Se-Cysteine is not possible as it doesn't possess correct tRNA for selenoenzyme synthesis. So, it must be reduced to selenide which would be used in the Se-Cysteine-tRNA formation (Weiss 2005). Selenomethionine, after absorption, could enter 'general methionine pool', thus, non-specifically incorporating into different body proteins providing a means of reversible selenium reserves which can be later utilized as an endogenous selenium supply for synthesis of selenoenzymes (Schrauzer 2003; Burk and Hill 2015). The selenium homeostasis in body is primarily achieved by the reserves of selenomethionine in kidney and liver. Before these reserves could be used in the biosynthesis of specific selenoproteins, selenomethionine must be catabolized to Se-Cysteine-tRNA for insertion into the active site of selenoenzyme (Weiss and Hogan 2005). Metabolism of hydroxy-selenomethionine, a precursor of selenomethionine, is also similar to that of selenomethionine (Surai et al. 2017).

To put it briefly, dietary intake of protein (quantity and quality) and its influence on microbial protein synthesis would impact the microbial incorporation of different selenium sources in the rumen. Also, inorganic selenium sources are exclusively associated with the production of selenoenzymes whereas fate of organic sources is split into two pathways viz. specific production of selenoenzymes and non-specific incorporation into different body proteins containing methionine/cysteine. It also highlights the build-up of greater body reserves of selenium with organic supplementation which would be beneficial to animal in times of stress or high demand (Gong and Xiao 2018; Gong and Xiao 2021).

Apart from the body tissue reserves, dairy cattle also deposit absorbed selenium into milk protein (casein) (Weiss and Hogan 2005). It is generally agreed that mineral status of dam is vital in determining the status in its offspring (Hostetler et al. 2003). Hair selenium content could also be used as long-term indicator of animal selenium status (Weiss et al. 1990). Other routes through which selenium leaves the body include feces, urine and exhalation. In ruminants, owing to their ruminal metabolism, major excretory pathway for dietary selenium is feces instead of urine which is major route in non-ruminants. Endogenous losses such as intestinal sloughing along with secretions of bile, pancreas, plasma, and saliva should also be accounted in fecal excretion when estimating true absorption of the mineral (Cruickshank 2021).

The presence of antagonists in the dietary milieu further complicates the circuitous route of selenium metabolism in ruminants. Many nutrient interactions involving vitamin E, sulphur, proteins, lipids, ascorbic acid and various microelements influence animal selenium requirements. Sulphate is less likely to hinder selenomethionine absorption as compared to inorganic supplements (Weiss and Hogan 2005), suggesting the plausibility that known antagonists of selenium might not treat the organic and inorganic supplements as same. Additionally, some other minerals have also been reported interfering with selenium absorption viz. copper, calcium, arsenic and cobalt (Goff 2018). Some intrinsic factors present within certain feedstuffs (i.e., soybean meal, millet, alfalfa) as well as the ratio between concentrate and forages also affect selenium availability to ruminants. Diversity of microbial populations and variation of the rumen fermenting environment with roughage and concentrate diets could be attributed to these manipulations (Spears 2003; Goff 2018).

Dietary supplementation of selenium to animals always results in a net gain of the element in environment. Therefore, caution should be taken to avoid disturbances in different agricultural production systems (Walker et al. 2010).

Feed (nutrient) Digestibilities

Major health beneficial role of selenium is indebted to its antioxidant properties. Similarly, development and operation of rumen is greatly obligated to its microbial efficiency. Research showed that it is possible to raise the antioxidant status of the ruminal microbiome using Se which results in increased microbial proliferation and rumen functioning (Arshad et al. 2021).

Most of the literature strongly agreed that neither the inclusion rate nor the source of selenium affected the feed intake in cows (Wang et al. 2009; Gong et al. 2014; Meyer et al. 2014; Juniper et al. 2019). Furthermore, there are reports of increased digestibilities of dry matter, organic matter, fiber (NDF and ADF), crude protein and crude fat with the addition of selenium in dairy cattle diets (Wang et al. 2009; Wei et al. 2019). Supplementary selenium also exhibited modifications in the ruminal fermentation patterns viz. acceleration in the total volatile fatty acid production especially propionate, and increased consumption of ammonia N by microbes, thus, suggesting improvement in the microbial fermentation rate in the rumen (Wang et al. 2009). On top, high proportions of propionate may potentially be instrumental in gluconeogenesis in dairy cattle during post-partum period (Gong and Xiao 2021), thus improving nutrient metabolism and responding to the challenge of negative energy balance.

In summary, via enhanced activity of microbes or enzymes, supplementation of selenium either inorganic or organic contributes to the act of fermentation in rumen coinciding with improved nutrient digestibilities which might reflect in better growth or enhanced milk yield in dairy cattle.

Selenium Effects on Reproduction

Animal body redox homeostasis is crucial to its metabolic health, especially for high producing cattle during periparturient period (Sordillo 2013; Zhang et al. 2021), and any disturbance in the antioxidant status may led to pathogenesis of different diseases. Selenium is reported to aid in curtailing systemic inflammation response (via downregulation of inflammatory phase proteins) as well as in the optimization of the lipid metabolism (via promotion of glycogenesis) during and after parturition in dairy cattle (Ren et al. 2020; Gong and Xiao 2021).

Supply of adequate selenium to dairy cattle during peri-parturient period may enhance their reproductive performance via better uterine health, prevention of retained placenta, and reduction in open days and number of services per conception (Wilde 2006; Silvestre et al. 2007; Machado et al. 2013). Pre-partum selenium supplementation is also stated to improve cow fertility by avoiding early embryonic deaths, whilst no effects on gestation length are reported. Selenium feeding pre-partum is also linked to improved progesterone levels in cows (Kamada 2017; Ullah et al. 2019) which might be a factor avoiding early embryonic losses.

Inadequate or marginal selenium supply could result in low reproductive performances in dairy cattle i.e., higher incidences of uterine infections, retained placenta, ovarian cyst, anestrous, stillbirth, abortion etc. In case of male cattle, selenium shortage or excess may affect spermatogenesis, resulting in poor semen quality and even infertility (Ahsan et al. 2014).

Milk Biofortification with Selenium Supplementation and its Effect on Milk Yield and Composition

In attempts regarding production of milk biofortified with selenium, plenty of literature is available detailing the superiority of organic selenium over inorganic form (Weiss and Hogan 2005; Wang et al. 2009; Calamari et al. 2010; Walker et al. 2010; Meyer et al. 2014; Séboussi et al. 2016; Zhang et al. 2018). Data on bioactivity of organic selenium showed more efficient transfer of ingested selenium to milk attributable to its retention in milk protein (selenomethionine replacing methionine) via non-specific route. This inevitably amplify the importance of differentiating between the selenium forms used and its dosage rate in the animal ration. In addition to selenium sources, physiological condition of cow is also vital to influence the milk Se levels (Burk et al. 2001).

Literature also showed improvement in milk yield (Juan Eulogio et al. 2012; Li et al. 2019) and modifications in milk components including fat, protein, lactose (Li et al. 2019; Zhang et al. 2020) when dairy cattle were supplemented with different selenium sources.

Selenium distribution among different milk components is also important. Data showed that selenium is abundantly distributed in milk protein i.e., 55-75% in casein, 17-38% in whey, and around 7% in fat (Van Dael et al. 1991). Research also showed that selenium supplementation has no or little effect on selenium distribution pattern in milk components (Muñiz-Naveiro et al. 2005). Thus, it can be deduced that removing fat from the milk (skimmed milk) would have little effect on selenium concentration of milk.

Deficiency and Toxicity

Selenium deficiency is much dreaded geographically when compared with its toxicity (Khanal and Knight 2010). Generally, dietary intake of selenium lower than 0.05 mg/kg DM in both humans and animals is perceived as insufficient. In ruminants, severe selenium deficiency can trigger nutritional myopathies i.e., white muscle disease, primarily in young calves and selenium-responsive ill-thriftiness. In adult dairy cattle, it is very common to be inflicted with marginal selenium levels which predispose them to various maladies viz. enhanced oxidative susceptibility (reduced antioxidant capacity, poor response to oxidative stress), poor thyroid hormone regulation, inefficient feed utilization, impaired growth, impaired immune function, reproductive disorders (cystic ovaries, metritis, infertility, early embryonic death, retained fetal membranes, high calf mortality, increased number of services per conception, low pregnancy rates), and compromised udder health (clinical and subclinical mastitis, higher milk somatic cell counts, reduced milk production). It is of the essence that proper assessment of body selenium status be made via various indicators (milk, blood, tissue selenium levels) before treating the animals to avoid toxicity (Silvestre et al. 2007; Sordillo 2013; Hendriks and Laven 2020; Ullah et al. 2020; Arshad et al. 2021).

Although, selenium had a profound and lengthy history of toxicity, its occurrence in current livestock setup is uncommon by virtue of proper management practices. Nevertheless, liable to human error, faulty selenium supplementation culminating into excessive intakes by animals can lead to the toxicity syndrome (selenosis). Signs of selenosis may appear with prolonged ingestion of selenium ranging between 5 to 50 mg/kg DM (Kaneko et al. 2008). National Research Council (2005) recognized 5 mg selenium/kg feed (DM basis) as the maximum tolerable level for ruminants. Factors affecting the selenium toxicity may include type of diet, animal sex and species, selenium form and supplementation duration (Arshad et al. 2021). Selenosis is also known as “alkali disease” (chronic) or “blind staggers” (acute) (Zarczynska et al. 2013). Selenium toxicity in livestock is characterized by lethargy, emaciation, loss of hair, roughness of coat, aching and sloughing of hooves, stiffness and joint related lameness, atrophy of the heart, liver cirrhosis, anemia and teratogenic effects along with abortions/stillbirths (Schöne et al. 2013). Apart from incorrect supplementation, selenosis could be an outcome of ingesting ‘selenium accumulator plants’ viz. *Astragalus*, *Atriplex*. In modern dairy, it is very unusual for animals to develop selenosis. Quite contrarily, the bigger task is getting enough to stay healthy and perform optimally (Schöne et al. 2013).

Conclusion

Feeding dairy cattle with supplementary selenium (0.3-0.5 mg/kg DM) is undoubtedly valuable ensuring the optimum intake of this micro element by the animal especially during the periparturient period. A great deal of scientific data showed selenium to be an indispensable element in dairy nutrition promoting the health status of animal viz. improving gut health, regulating immune system, balancing thyroid hormones, contributing to disease resistance, boosting reproductive and productive performances. Moreover, the supplemental selenium potentially enhances the quality of final food product. Organic forms of selenium have been shown to be superior in terms of availability and execution of selenium-dependent responses. Usage of selenium biofortified forages in dairy diets has also been tried and proved beneficial but it is tied to the regional legislature. The overall selenium body status, calculated via different indicators (milk, blood, tissue etc.), assist the nutritionists in maintaining a delicate balance between the dark side (toxicity) and the bright side (productivity) of the selenium. The supra-nutritional doses of organic selenium in dairy cattle diets could aid in the production of functional food, however, its long-term effects on the trichotomy of animal, human and their environment needs further research.

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CRISPR CAS-9 Mediated Genome Editing and One Health Related Diseases

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INTRODUCTION

One Health Concept

The concepts of One Health began in the late 19th and 20th centuries, firstly as One Medicine, then as One World and finally as One Health (Atlas 2012) with the contributions of Rodulf Virchow, known as the “Father of comparative medicine, cellular pathology and veterinary pathology” and Willian Oster, known as “Father of Modern Medicine”. The most commonly used definition suggested by the One Health Global Network is: “One Health recognizes that the health of humans, animals and ecosystems are interconnected. It involves applying a coordinated, collaborative, multidisciplinary and cross-sectoral approach to address potential or existing risks at the animal-human-ecosystems interface” (Mackenzie and Jeggo 2019).

Significance of One Health

The significance and importance of one health have increased enormously over the past three decades. It has been observed for a long time that the majorities of novel and newly emerging infectious diseases were zoonotic and originated in animals, particularly wildlife. Such diseases are primarily linked to human interruption and activities related to ecosystems, land use, agriculture intensification, suburbanization, industrialization, deforestation and international trade. These factors make one health highly important to make sure that the health of people, animals and the environment is safe. These are interconnected to make one health triad (Atlas 2012) (Fig. 1).

The one health concept is based on the early detection and timely diagnosis and treatment of emerging zoonotic diseases and their prevention and control. Many diseases have a significant social impact in poor, developing or underdeveloped countries. It also leads to antimicrobial resistance (AMR), because resistance develops as a result of misuse or under usage of antimicrobials which may develop in human beings, animals and the environment and transfer from one to the other (Mackenzie and Jeggo 2019).

Emerging and Endemic Zoonotic Diseases:

Emerging and endemic zoonotic diseases have devastating consequences to human health in the past and these damages are still present (Table 1). Many emerging infectious diseases have emerged and spread worldwide like Zika, Ebola, Congo fever, Swine flu, SARS and MERS, and then the COVID-19 pandemic. In recent years, it has been seen that several zoonotic diseases have emerged and spread in both animals and humans. More than 6-70% of human infectious diseases are zoonotic and have an animal origin. Similarly, out of five newly emerging human diseases each year, three diseases have animal origin. In spite of the great deal of research, infectious diseases like tick borne diseases, anthrax, TB, brucellosis, Coronavirus infection and rabies are still threat to human health and welfare (Atlas 2012). A target based research like target oriented genome editing (discussed in detail in the later parts of the chapter) is much needed to tackle the problem of diseases related to one health as per mentioned in Table 1.

Genome editing technique and One Health

The development of genome editing technology in the 1970s marked the beginning of a new era for biology. Genome editing technology came in action from the mid-era of the 20th century. Bio-engineering technology involves knockout, knock-in, and replacement of specific section of genomic sequences resulting in controlled and

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desired alteration in a eukaryotic cell. It has revolutionized the human way of perceiving things regarding welfare and survival (Gaj et al. 2016). Pronuclear micro-injection, developed in the 1980s, was the one of the principal technique exercised for genetically modified organisms (Gordon et al. 2020). The process includes the transfer of

Table 1: Summary of various one health related diseases caused by viral, bacterial and parasitic pathogens

Pathogen Type	Pathogens	Natural Host	Mode Of transmission	Disease caused	References
Viral Agent	Lyssaviruses	Dog, Bat, Cat, Foxes	bites or scratches of infected animal	Rabies	(León et al. 2021)
	West Nile Virus	Birds	Culex species of Mosquitoes	West Nile Fever	(Petersen et al. 2013)
	SARS-CoV-2	Bats	direct, indirect, or close contact with saliva or respiratory droplets of infected people	Respiratory Track Disease	(Yang et al. 2020)
	Monkeypox virus	monkeys, anteaters, hedgehogs, rodents	direct contact (bites, scratches, slaughtering) with infected animals	pimples or blisters throughout the body (Similar to smallpox)	(Pastula and Tyler 2022).
	Ebola virus	Bat or nonhuman primate	Direct contact with an infected animal , contagious	Ebola hemorrhagic fever	(Groseth et al. 2007)
	Zika Virus (Flaviviruses)	Nonhuman and human primates	Aedes aegypti and Aedes albopictus	Guillain-Barré syndrome, microcephaly	(Musso and Gubler 2016)
	Hanta Virus	Rodents	Direct or indirect contact with rodent’s urine, droppings or saliva	Human Pulmonary Syndrome (HPS), Hemorrhagic fever renal syndrome (HFRS)	(Muranyi 2005)
	Swine Influenza (influenza virus H1N1)	Pigs	Asymptomatic carrier pigs or Pig aerosols	Respiratory disease in humans	(Olsen 2002)
	Avian Influenza Virus	Domestic Poultry or Water Bird	Direct contact between infected bird’s feces, mouth or nose secretions.	Respiratory track Infection	(Lee and Saif 2009)
Bacterial Agent	Bacillus anthracis	sheep, cattle, horses and goats	Direct or indirect contact with infected animal	Anthrax	(Sidwa et al. 2020)
	Borrelia burgdorferi	mice, chipmunks, raccoons, squirrels, lizards, White Footed Mouse	ticks	Lyme Disease	(Bernard et al. 2019).
	Yersinia pestis	Rodents	Fleas	Plague	(Prentice and Rahalison 2007)
	Brucella spp.	Goats and sheep (undercooked meat or consuming unpasteurized /raw dairy products)	meningitis and endocarditis	Brucellosis	(Karponi et al. 2019)
	Salmonella sp.	Domesticated or/and wild animals (poultry, cattle, dogs, rodents, swine and cats	Food or water contaminated by feces	Gastroenteritis, diarrhea, tyohoid and enteric fever	(Ajmera and Shabbir 2022)
Parasitic Agent	Giardia duodenalis	Rodents, cows, and sheep	Direct or indirect contact with contaminated water, food, surfaces, or objects	Giardiasis	(Sprong 2009)
	Schistosoma mansoni, S. haematobium, and S. japonicum	snail	Drinking Contaminated water containing parasite eggs	Schistosomiasis	(Gryseels et al. 2006)
	Toxoplasma gondii	Cats	Eating undercooked contaminated meat, exposure from infected cat feces	Toxoplasmiosis	(Remington et al. 2004)
	Trematodes	Cats, dogs, foxes, pigs and rodents	Consumption of water and food contaminated with trematodes in the larval stages.	Trematodiasis	(Keiser and Utzinger 2005).
	Ticks (Ixodid Tick)	Cattle, sheep and goats.	Contact with infected ticks or animal blood	Crimean-Congo Hemorrhagic Fever	(Whitehouse 2004).
	Trypanosoma brucei	Domestic cattle	tsetse fly, Glossina sp.	Trypanosomiasis	(Brun et al. 2010)

foreign DNA into the nucleus of a fertilized egg which develops into a transgenic animal. The initial trial of zygote microinjection for producing transgenic animals like pigs, sheep, and rabbits was carried out in 1985 (Navarro-Serna et al. 2020). In the late 1990s, Somatic Cell Nuclear Transfer (SCNT) further modifies genome editing technique by facilitating the transfer of the nucleus from a somatic cell into the enucleated fertilized egg cell. The transfer results in the production of an organism genetically identical to the enucleate nucleus (Gouveia et al. 2020).

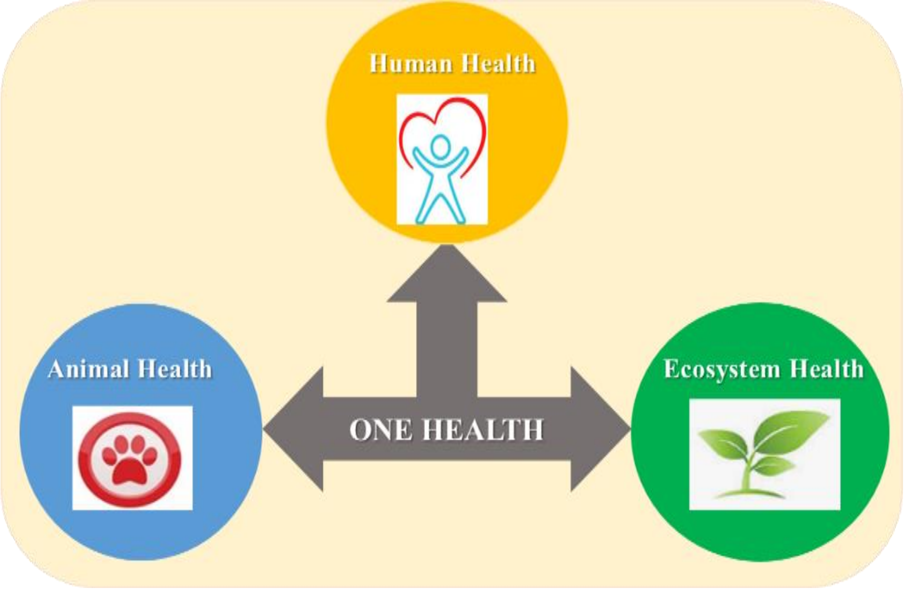


Fig. 1: The One Health Triad comprises of three components including human health, animal health and ecosystem health.

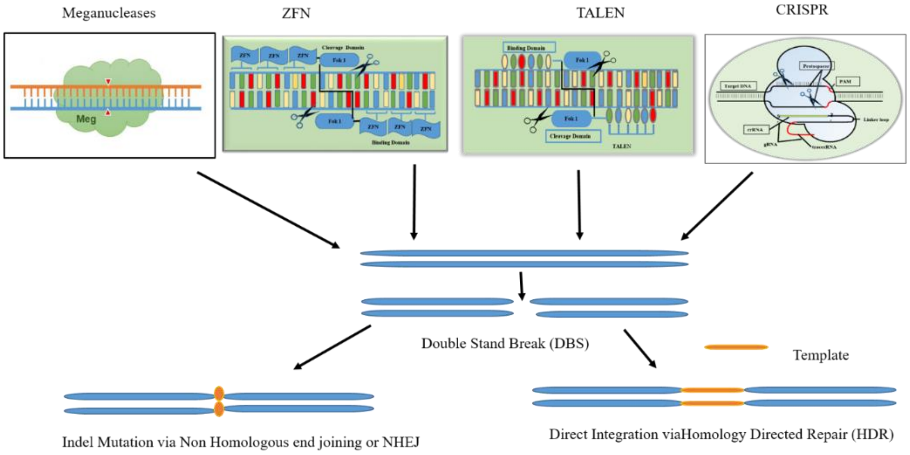

Meganucleases
ZFN
TALEN
CRISPR
Meg
Double Stand Break (DBS)
Template
Indel Mutation via Non Homologous end joining or NHEJ
Direct Integration viaHomology Directed Repair (HDR)

Fig. 2: Repair mechanisms of Gene Editing Techniques including meganucleases, zinc finger (ZF) nucleases, transcription activator-like effectors nucleases (TALENs9) and CRISPR-Cas9 system.

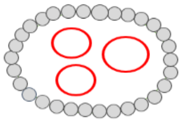
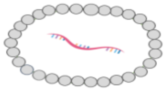
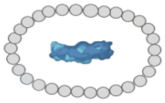
DNA double strand breaks (DSBs) is considered the most effective way to do site specific genome editing Four major classes of DNA-binding proteins have been engineered so far to achieve target site based genome editing which includes meganucleases, zinc finger (ZF) nucleases, transcription activator-like effectors (TALEs) and most recently the RNA-guided DNA endonuclease Cas9 from the type II bacterial adaptive immune system CRISPR. These molecules are also referred as molecular scissors and follow more or less the same type of mechanism of genome editing (Khalil 2020).

Initially, these molecules identify the targeted sequence, bind with it and induce a DSB at that particular position where editing is required (West and Gill 2016). Endonucleases go either through non homologous end joining (NHEJ) or homology directed repair (HDR) mechanisms to restore DSB (Fig. 2). Ninety percent of restoration occurs through NHEJ because it can perform its activity during all phases of the cell cycle except in the M phase. Without a repair template, NHEJ undergoes linkages of disconnected ends straightway, resulting in insertion or deletion (indel) at the DSB. Whenever a mutation occurs at that particular site and causes knockout/in of gene and loss/gain of function, it is error prone. While HDR, along with a repair template, will join similar sequences containing dsDNA breaks and result in targeted modifications at the desired locus (Petersen et al. 2013). Furthermore, NHEJ is active during cell cycle (G1 – S–G2/M), whereas HDR pathway is only active during the S and G2/M phases (S>G2/M) (Ranjha 2018) as mentioned in Table 2.

Meganucleases

The technique is based on identifying target site, and then cutting it using endonucleases activity. The benefit of using the Meganucleases technique is its less toxicity towards cells because it is naturally present in cell. In contrast, drawback of this method is less or accurate interaction between meganucleas-protein and target DNA sequence specificity which lessens its use as genome editing technique (Zaslavskiy et al. 2014).

Table 2: Delivery Strategies for CRIPR-CAS9 system

Delivery method	Characteristics	Limitations	References
Delivery of CRISPR-Cas9 via plasmid 	-Simplest and Cost effective way. -Delivery of Cas9 and gRNA via two plasmid or integration of gRNA expression on the same Cas9 plasmid.	-Greater chances of off target effect due to longer expression time. -Time consuming due to transcription and translation. Contains the potential for insertional mutagenesis.	(Chen et al. 2020)
Delivery of CRISPR-Cas9 via mRNA 	-Faster and easier than plasmid DNA. -Transcription not needed. -Short lived Cas9 expression so minimum chances of off target effect.	-It can only be used to express Cas9 for a short period of time due to mRNA instability and susceptibility to RNase degeneration.	(Humphrey and Kasinski 2015)
Delivery of CRISPR-Cas9 via protein 	-Gene editing efficiency is higher than that of DNA and mRNA. -Lesser chances of off target effect due to transient Cas9 expression.	-Costly as compare to DNA and mRNA. -Illicit immunologic responses due to Delivery via bacterial origin protein.	(Liang et al. 2015)

Zinc Finger Nucleases (ZFNs)

ZFNs are not naturally occurring nucleases and can be generated by joining restriction endonucleases with ZF protein providing a very specific target site cutting. This technique has the advantage over meganucleases as it is simple and specific (Ochiai and Yamamoto 2017). However, assembling zinc finger domains to bind an extended array of nucleotides with high affinity has not proven easy. Despite improving this technique with advancements in research, ZFPs binding specificity with desired targets remains a significant challenge (Ely et al. 2021).

Transcription activator-like effector nucleases (TALEN)

TALENs resemble ZFNs in terms of formation of DNA binding protein and endonucleases complex and mode of action. Instead of using ZF, another DNA-binding protein domain called TAL effector is used to bind with endonucleases (Hensel and Kumlehn 2019). TALENs are a little more specific because these can target even a single nucleotide unlike ZFNs where at least three nucleotides can be targeted (Chandrasegaran and Carroll 2016). However, the limitations of both techniques are almost the same, which are mentioned above in ZFNs (Juillerat et al. 2014).

Clustered Regularly interspaced short palindromic repeats (CRISPR)

The first hint of CRISPR manifestation was reported in 1987 from a unique repeating DNA sequence found in *Escherichia coli* while studying phosphate metabolism genes. Clustered regularly interspaced short palindromic repeats (CRISPRs) were identified in archaea in 1993, notably in *Haloferax mediterranei* (Ishino et al. 2018). The CRISPR/Cas-system complex an ancient immune (acquired) system of some bacteria and archaea species actively protecting against bacteriophage DNA. The initial application of CRISPR on the mammalian genome was started in 2013 (Cong et al. 2013). The short time line of CRISPR CAS9 development is shown in Fig. 3 (Khurshid et al. 2018).

Structure of CRISPR-Cas9 system

CRISPR I made up o two RNAs; CRISPR RNA (crRNA) and tracer RNA (trans-activating RNA). Both crRNA (spacer + palindromic repeats) and tracrRNA are distinct entities. The crRNA gives Cas9 target specificity, but it cannot attach to Cas9 directly. These two portions (crRNA + tracrRNA) are joined by a linker to produce a single molecule known as single-guided RNA (sgRNA), which can be synthesized in the laboratory. sgRNA binds to the Cas protein, resulting in the creation of the Cas Complex. CRISPR also contains spacer DNA, 26 to 72 bp short sequence segments, homologous to phage or plasmid DNA. Each spacer is separated from another spacer through repeat sequences of similar size (Jiang and Doudna 2015).

Cas9 can be directed to break dsDNA at any location on the genome specified by the guide RNA sequence and a Protospacer Adjacent Motif (PAM). The PAM is a critical targeting component to protect the CRISPR domain from attack. A 5′-NGG-3′ PAM is a widely used sequence, derived from *Streptococcus pyogenes* (SpCas9) (Cong et al. 2013). Basic structural framework of CRISPR is shown in Fig. 4.

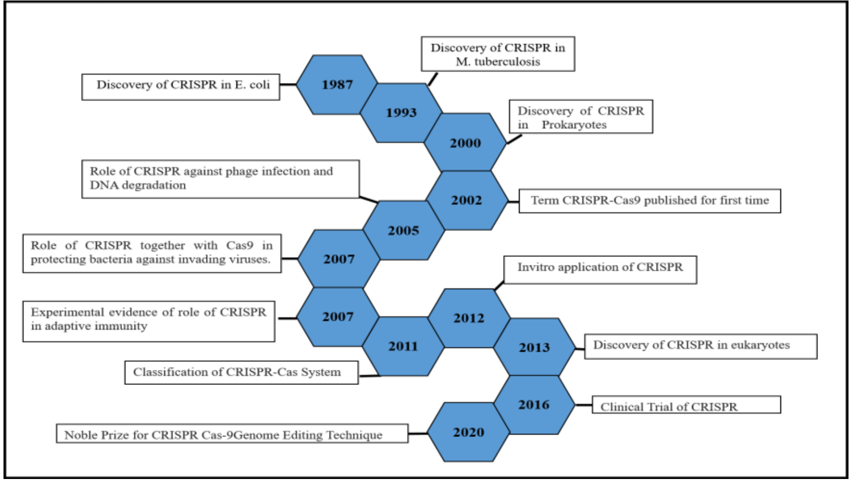

Discovery of CRISPR in M. tuberculosis
Discovery of CRISPR in E. coli
1987
1993
Discovery of CRISPR in Prokaryotes
2000
Role of CRISPR against phage infection and DNA degradation
2002
Term CRISPR-Cas9 published for first time
2005
Role of CRISPR together with Cas9 in protecting bacteria against invading viruses.
2007
Invitro application of CRISPR
Experimental evidence of role of CRISPR in adaptive immunity
2007
2012
2011
2013
Discovery of CRISPR in eukaryotes
Classification of CRISPR-Cas System
2016
Clinical Trial of CRISPR
Noble Prize for CRISPR Cas-9Genome Editing Technique
2020

Fig. 3: A brief timeline of CRISPR technique development

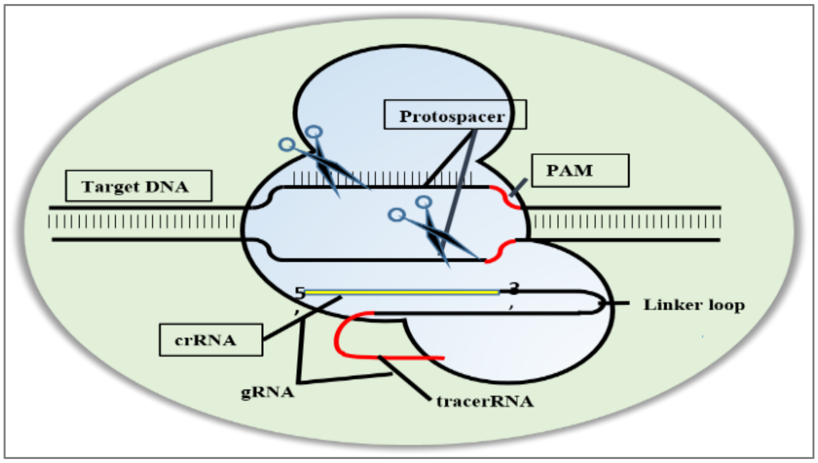


Fig. 4: Schematic diagram of Structural Framework of CRIPR-Cas9 system

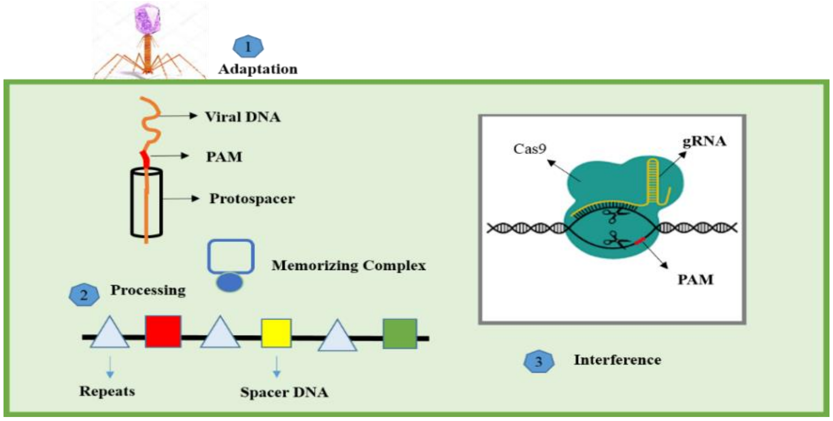


Fig. 5: Mechanism of CRISPR Working

How does CRISPR-Cas system work?

The immune response to CRISPR-Cas consists of three phases; adaptation, expression and interference as shown in Fig. 5.

Adaptation phase: After detecting a discrete, short pattern PAM, a separate complex of Cas proteins attaches to a target DNA. The adaptation complex inserts the protospacer

DNA into the array after duplicating the repeat at the 5' end of the CRISPR array and converting it to a spacer.

Expression phase: The pre-CRISPR RNA (pre-crRNA) is processed into mature crRNA, containing the spacer sequence and parts of the flanking repeats.

Interference phase: A sequence of amino acids is inserted as a guide for identifying the protospacer in a virus or plasmid's invading genome, cleaved and down regulated by a Cas nuclease/s (Lander et al. 2016).

Delivery System for CRISPER Cas9

In the type II CRISPR Cas system, the crRNA effector complex is a single multi-domain protein called Cas9. Cas9 signature gene alone is insufficient for categorizing it as a specific CRISPR gene hence Cas1 and Cas2 genes are used as additional genes for type II system identification. Cas9 requires two accessory nuclease domains to cleave the target DNA, HNH, and Ruv C-like, and each nuclease only cleaves one strand of target DNA (Krzysztof 2014).

CRISPR CAS9 protein has a molecular weight of 160 kDa, and the long phosphate backbone of the sgRNA provides a net negative charge to the complex when it forms a ribonucleoprotein (RNP) complex which makes CRISPR delivery challenges Both the vehicle (CRISPR-Cas9) and the cargo (CAS nucleus and guide RNA) are involved in the CRISPR-Cas9 delivery. Delivery can be performed via DNA, mRNA, or proteins as shown in Table 2 (Jinek et al. 2014).

CRISPR Cas-9 and its application in One Health related zoonotic diseases

CRISPR Cas system has huge number of applications because it is simple to use and works in a precise manner. It has multiple applications in diagnosing and treating of diseases including one health-related diseases. Some of those are discussed as follows:

Bacterial Diseases:

Anthrax

Anthrax, caused by *Bacillus anthracis (B. anthracis*) is a lethal zoonotic disease with a very high mortality rate. *B anthracis* spreads by spores which have been recognized as a bioweapon (Sidwa et al. 2020). Various studies have shown that CRIPR Cas technique can reduce this organism's virulence and pathogenicity (Inglesby et al. 2002; Wang et al. 2021; Choate et al. 2021).

B. anthracis possesses two plasmids responsible for virulence including pXO1 and pXO2. The pXO1 encodes anthrax toxin proteins; protective antigen (PA), the lethal factor (LF) and the edema factor (EF). The pXO2 plasmid encodes for the capsule. Researchers constructed a CRISPR system consisting of scissors plasmids which deliver via a shuttle plasmid and target virulent toxin protein genes. A 20nt long sequence guiding the Cas9 nuclease is used as scissors to break the target DNA. The study has shown that these two plasmids had a key role in virulence of *B. anthracis*, and the loss of any one plasmid due to CRISPR-Cas system resulted in a great increase in the elimination rate of the organism (Wang et al. 2021).

Anthrax toxin receptor 2 (ANTXR2) is a membrane receptor of *B. anthracis*. In a study it has been seen that changes in the levels of expression of ANTXR2 were observed between human and non-human primates using CRISPR activation (CRISPRa). The study's findings revealed a complex evolutionary relation between human and non-human primates in ANTXR2 expression. In human immune cells, ANTXR2 was 8-fold down-regulated compared to non-human primates, indicating regulatory changes early in the evolution of modern humans. This study provided a model which showed the wadaptation of humans to anthrax disease. Along with other applications using the CRISPR-Cas system can also help in identifying the early ecological changes (Choate et al. 2021)

Brucellosis

Member of genus Brucella is.the causative agent of the Brucellosis. Most common species of *Brucella is B. melitensis*, and it mainly affects ruminants, causing reproductive track-related conditions like abortions and infertility. In humans, it causes severe clinical conditions like arthritis, endocarditis, meningitis, infertility and abortion in women. The treatment against brucellosis is usually not very effective, and currently no effective vaccine is available (Karponi et al. 2019).

In a study, *B. melitensis* infected ovine macrophages were used to simulate the host cell and pathogen's interaction *in vitro*. Lentivirus vectors with CRISPR-Cas9 system to target Brucella RNA pol A gene was transduced into the infected cells. By targeting the RNA pol A gene bacterial load per cell was reduced significantly (Karponi et al. 2019).

Xu et al (2022) have developed a system to detect different Brucella species in infected blood and milk using the CRISPR-Cas12a system with recombinase polymerase amplification (RPA). This platform uses CRISPR-Cas12a-RPA fluorescent biosensor and CRISPR-Cas12a-RPA electrochemical biosensor which could detect 2-3 copies of plasmid DNA and can help in the early diagnosis or detection of brucellosis.

Lyme Disease

Lyme disease is a tick-borne infection caused by the bacterial spirochete *Borrelia burgdorferi* and is transmitted by the bite of an *Ixodes* genus tick (Bernard et al. 2019).

White-footed mice are the main reservoir of the *Borrelia* bacteria that causes the disease. It is a zoonotic disease and can be transmitted from mice to humans through the bites of black-legged ticks. If untreated, disease progression may lead to early arthritis, neurologic manifestations and cardiac involvement problems (Norris 2018).

Gene editing progress in ticks has been slow, mainly because ticks coat their eggs in large amounts of wax, preventing the successful injection of CRISPR components into embryos at the correct developmental stage without destroying the egg (Sharma et al. 2022). Therefore, instead of targeting the disease vector, researchers have used CRISPR to prevent tick-borne disease by using CRISPR-based genome editing to heritably immunize the white-footed mice responsible for infecting many different types of ticks. Introducing antibody-encoding resistance alleles into the local mouse population was anticipated to disrupt the disease transmission cycle for decades. To achieve this, engineered mice were released in the natural population to breed with natural mouse populations. In this way, the off springs can be immunized without gene drive (Buchthal et al. 2019).

Viral Diseaes

Rabies

Rabies is acute, progressive encephalitis caused by a lyssavirus. These viruses are bullet-shaped, single-stranded, negative-sense RNA viruses. The reservoir hosts are known to be bats. Most mammalian species, including human beings, are susceptible to this disease. The virus transfers to the susceptible host via direct transmission through infected animal bites and transdermal inoculation of virus-laden saliva (León et al. 2021).

Currently rabies treatments primarily focus on mass vaccination and *post-exposure prophylaxis (*PEP). However, tools like gene therapy or gene editing are needed to treat or control rabies in the future. A CRISPR-Cas9 system in combination with (Induced pluripotent stem cells) iPSCs can make corrections *in vitro* in genes. Gene delivery tools can be used later to deliver the corrected/edited genes to the target organs. In the same way gene therapy based on the CRISPR-Cas9 system in combination with the Microhomology-mediated end-joining method can also stop/eliminate rabies virus in the infected cells (neurons). This technique can also be used to treatment rabies even after signs appear (Nelwan 2018).

Coronavirus Disease 19 (COVID-19)

Coronaviruses (SARS-CoV-2) belong to the Coronaviridae family in the Nidovirales order. The Respiratory system is the primary target of SARS-CoV-2; however, it also can infect other organs/systems. Due to COVID19 pandemic a large number of human causalities occurred. This disease is also zoonotic and known to be transmitted from bats (Yang et al. 2020).

Scientists have developed a host-pathogen protein-protein interaction map for SARS-CoV-2 and other coronaviruses. This interaction network identified hundreds of relevant proteins and then used high-throughput CRISPR gene knockout to functionally validate each of these. CRISPR has massively accelerated both the search for novel drug targets for treating infectious diseases and the repurposing of existing drugs to treat emerging pathogens. For example, CRISPR knockout of a key enzyme in the fatty acid metabolism pathway, fatty acid synthase (FASN), demonstrated its role in SARS-CoV-2 infection and replication and suggested existing inhibitors of FASN could prevent infection (Gordon et al. 2020).

Human Papillomavirus (HPV)

Human papillomavirus (HPV) is a small DNA virus known to cause cervical and other cancers. Researchers have identified the key genes of HPV responsible for oncogenesis; E6 and E7, leading to these genes being targeted with CRISPR as a potential therapeutic tool for HPV. For example, several studies have confirmed that targeting the E6 and E7 genes with CRISPR-Cas9 leads to decreased levels of the proteins, apoptosis of infected cells, and growth inhibition (Zhen and Li 2017).

Pre-clinical studies used stealth liposomes to deliver CRISPR-Cas9 editing components to target the E7 gene in HPV16 tumors in mice. The treatment effectively cleared the tumors without causing toxicity to the liver or spleen of the mice. Positive results like these led to clinical trials of this therapy being tested in human patients (Jubair et al. 2021).

Human Immunodeficiency Virus (HIV)

Acquired immune Deficiency Syndrome (AIDS) is still a major global health problem. It is caused by Human Immunodeficiency Virus (HIV) infection and destroys host’s immune system (Bowers et al. 2014). Due to the latent nature, it is challenging to eliminate this virus even after highly active antiretroviral therapy (HAART). To overcome this challenge, the CRIPR-Cas9 trial has been initiated which, up until now, are providing satisfactory results regarding HIV treatment (Xiao et al. 2019). Experiments have been conducted to target the HIV-1 genome using the CRISPR-Cas9 guide RNA to target conserved sites in the LTR-U3 region. The results demonstrated inactivation of viral gene expression and restriction of virus replication in different HIV-1 latently infected cell lines including the T cell line, pro-monocytic cell line, and microglial cell line. It has also been observed that genotoxicity, and off-target effects were very little (Hu et al. 2014; Lebbink et al. 2017). Recently, the employment of CRISPR-Cas9 and gRNA of Staphylococcus aureus in lentiviral vector have been shown to excise latent HIV-1 provirus and suppress proviral reactivation (Wang et al. 2008).

West Nile Virus

West Nile virus (WNV) is a member of the Flaviviridae family. It is an enveloped single-stranded RNA virus. Mosquitos are mainly responsible for transmitting this pathogen to humans (Petersen et al. 2013). WNV causes a neurological infection which leads to massive neuronal cell death. Seven genes were found to be involved in WNV-induced cell death. These genes, including *EMC2*, *EMC3*, *SEL1L*, *DERL2*, *UBE2G2*, *UBE2J1* and *HRD1* were identified using CRISPR-Cas9 based identification method. By disrupting these genes protection against WNV-induced cell death in three cell lines was conferred. Interestingly replication of WNV was not blocked even after knocking out of these genes which proved that these genes were essential for the pathways involved host cell death (Ma et al. 2015).

Parasitic Diseases

Malaria

Malaria is caused by a *Plasmodium* parasite and is transmitted by *Anopheles gambiae* mosquitoes. Several gene drive systems have been developed to control mosquito -borne diseases using genome/gene-edited mosquitoes. CRISPR system was also used to make the male gene dominant over the female one by editing or modifying the sex-determining gene. This modification was then spread using a gene drive, which later helped to eliminate infected female mosquitoes from the population. It was observed that within 7-11 generations, the modified gene became 100% prevalent. egg production reduced gradually and the population totally collapsed within the laboratory environment (Hammond et al. 2016).

Similarly, the researchers studied the inactivation of the fibrinogen-related protein 1 (*FREP1*) gene. CRISPR-*FREP1* knockout mosquitoes exhibited a slower development in their pre adult life stage and were less likely to feed on blood meals. Those knock-out mosquitoes also laid fewer eggs with less viability (Dong et al. 2018).

Toxoplasmosis and Chagas Disease

Toxoplasma gondii, is an intracellular protozoan and is the etiological agent of Toxoplasmosis. It is also a zoonotic disease and most infections in humans are asymptomatic. However, in newborns, children and immune- suppressed individuals a significant disease can be produced through primary infection/ reactivation. Vertical transmission, organ transplantation, and ingesting of infected tissue cysts are main transmission routes. Improperly cooked/raw meat and contaminated food and water may have tissue cysts or oocysts, which can cause infection (Saadatnia and Golkar 2012).

Similarly, Chagas disease is also a vector borne disease caused by the parasite *Trypanosoma cruzi* and the vector for this disease is a reduviid bug. (Brun et al. 2010). Genome editing techniques were also employed for both of these parasitic pathogens. CRISPR-Cas9 high-throughput analysis was used to simultaneously knock out/knock down all nuclear protein-coding genes in *Toxoplasma gondii* (Nødvig et al. 2015). To achieve this guide RNA library constitutively expressing Cas9 was transfected into parasites. CRISPR-Cas9 was also used to silence the gene encoding GP72 (responsible for flagellar attachment) of *T. cruzi* (Lander et al. 2017). Similarly, in another study knockout of P21 gene of *T. cruzi* was performed using CRISPR-Cas9. As a result loss of expression of P21 was observed. It has also been observed that this process has a direct impact on the cell cycle. It caused the cessation of the cell cycle at the G1 phase which eventually slowed cell division and growth rate of epimastigotes (Teixeira et al. 2022).

Conclusion

Genome editing especially the CRISPR-Cas system has endless applications. Much work still needs to be done to eliminate zoonotic diseases and diseases related to one health. CRISPR based diagnosis and treatments need time. Nowadays focus of CRISPR based genome editing is on prevention and treatment of infectious diseases caused by bacteria and viruses of zoonotic importance. Safe, effective and less off-target effects are desired in CRISPR-based therapies. The most important lesson learnt in the COVID 19 pandemic, is to act proactively against zoonotic, newly emerging or re-emerging viral infections. However, due to higher mutability in viruses, conventional methods become less effective, hence focus should be diverted on the techniques like CRISPR to combat these challenges. New drugs, vaccines diagnostic techniques and products should be developed using CRISPR technology to reduce the damages caused by zoonotic infectious diseases.

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Plasma Membrane Camouflaged Nanoparticles: An Emerging Antibacterial Approach

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INTRODUCTION

Diseases caused by bacterial infections pose a major impact on both human and animal health and this threat is continuously increasing by enhanced antibiotic resistance (Carly et al. 2021). These bacterial infections may result in high rates of morbidity and mortality. Hence, emphasizing the urgent need to treat life threatening bacterial infections effectively. Recent developments in antibacterial treatment have mainly focused on targeted and responsive approaches for antibiotics and antibiotic alternatives (Wu et al. 2021).

Currently, new innovative approaches in nanomedicine hold promise for the treatment of bacterial infections, as the application of metal, metal oxide and polymeric nanoparticles dramatically promotes the therapeutic efficacy during infection (Baptista et al. 2018) as mentioned in Table 1.

A nanoparticle that has been administered into the body is destined to face a highly complex environment that is naturally capable of identify and eliminate foreign substances. For instance, the bloodstream contains various cellular and protein-based components, which may quickly impair the subscribed function of the nanoparticle (Haroon et al. 2022).

Furthermore, destruction by the reticuloendothelial system is one of the significant obstacles that almost all platforms must overcome before a nanoparticle can reach its target (Caliskan et al. 2019).

However, the difficulties associated with the application of synthetic nanoparticles divert the researcher's attention towards bioinspired nanotechnology which obtains its design cues from nature (Jiang et al. 2019).

Cell membrane coated nanoparticle based solutions are becoming increasingly popular in medical research, as they may offer significant benefits in terms of both efficacy and safety over current therapies and diagnostics (Islam et al. 2018).

Researchers are now working to exploit naturally-derived cell membranes as a way to deliver nanoparticles with enhanced bio interference capabilities, rather than attempting to replicate these biocompatible functions via synthetic techniques. This top-down approach is simple, versatile and has the potential to increase therapeutic efficacy as compared to existing nanocarriers significantly. Furthermore, introducing a natural membrane substrate on the surface of the nanoparticles has allowed additional applications beyond those traditionally associated with nano-medicine. Overall, there is still a considerable slot for improvement and development of new coated nanodrug moieties as researchers continue to improve existing workflows and discover new and exciting applications of this emerging nanotechnology (Fang et al. 2018). Ultimately, the goal is to create nanoparticles with surfaces that help them ignore everything but their targets, which is extremely difficult. Therefore, the addition of specific targeting mechanisms during the formulation of nanoparticles can improve efficacy by promoting preferential accumulation at the site of interest by reducing nonspecific targeting (Panda et al. 2020). The cell membranes coated nanoparticles.

Cell Membrane-coating Techniques

Membrane Extrusion

To produce a cell membrane-coated nanoparticle, the specific cell membrane must be coated over the nanoparticles core. The most frequently mentioned technique for cell membrane coating is membrane extrusion. It is one of the primary cell membrane-coating techniques. Biological membrane extrusion is performed so that the cell membranes and nanoparticles may move through membranes of various pore sizes concurrently, leading to the wrapping of the membranes over the nanoparticles. This method is beneficial; however, mechanical forces may affect the structure of the membrane (Hu et al. 2011).

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Table 1: Summary of potential antibacterial nanoparticles against specific species of bacteria

Nanoparticles	Antibacterial Mechanism	Characterization	Targeted Species	References
Silver nanoparticles	Damage cell wall structure	19 nm	E. coli	Bruna et al. 2021
PEGylated Liposomes	Accumulate Selectively Soft tissues infections	90-100 nm	*Staphylluscoccus aureus*	Laverman et al. 2001
Superparaegnative Iron Oxide Nanoparticles (SPIONPs)	Accumulate Selectively Soft tissues infections (lungs)	35nm	*Staphylluscoccus aureus*	(Kaim et al. 2002)
Cationic Peptide Nanoparticles	increase the local charge and mass densities of the bactericidal components (Electrostatic Interactions)	177±6 nm, 152±8 nm	Broad spectrum gram positive Bacteria, MRSA, yeast and *C. albicans*	(Liu et al. 2009)
polycarbonate-based block copolymer nanoparticles	Disintegration of cell membrane	0.2 um	Broad spectrum gram positive Bacteria, MRSA and fungi	(Nederberg et al. 2011)

Ultrasound

Consistent and stable cell membrane-coated nanoparticles (CMCNPs) can be obtained by ultrasound technique. This technique also encourages the membrane and nanoparticles to naturally form a core shell structure with a little loss of substantial material. However, it may destroy the nanoparticles (Copp et al. 2014).

Electroporation

In this technique, nanoparticles and cell membrane vesicles are first mixed in the microfluidic system with the S and Y-shaped channels. Then instantaneous pores are created on the vesicles by using electrical pulses at the exit of the channels and thus allowing nanoparticles to penetrate the vesicles (Rao et al. 2017).

Co-incubation

Another technique involves co-incubation of nanoparticles and live cells followed by the addition of serum-free media for secretion and production of exosomes. While wrapping the cell membrane over nanoparticles, it is vital to keep in mind that the coating process makes stable homogenous nanoparticles that do not interfere with the function of the nanoparticles or the cellular membrane (Silva et al. 2013).

Improving the Approaches of Membrane Extraction and Coating

Complete elimination of harmful intracellular proteins or organelles is necessary to obtain high purity and quality membranes; however, it is challenging to do so with the present methods of membrane extraction and wrapping over nanoparticles, which mainly include gradient centrifugation, sonication and mechanical extrusion. These might significantly restrict application; thus a new technique or technology must be created. For instance, microfluidic sonication and microfluidic viscoelastic flow technologies have demonstrated their unique ability to sort extracellular vesicles of high purity according to their size. Additionally, rapid and large -scale synthesis of functional membranes following standardized operation techniques is required to address clinical translational needs. Similar to earlier research, current technology cannot accomplish it well. The development of good manufacturing practice (GMP) standards for the clinical use of dendritic cells, stem cells and T cells has aided in the production of high-quality cell membrane-coated nanoparticles (Gurunathan et al. 2021).

Cell Membrane Protein Displaying Multifunctional

Membrane proteins are vital in maintaining cell signaling adhesion and mediating the effectiveness of medication administration and targeting for bioinspired membrane-coated nanoparticles. However, the cell lysis caused by extrusion, sonication, and centrifugation operations may disrupt specific critical proteins or membrane uniformity. Functional proteins may assist in improving targeting capacity and enhancing treatment efficacy. However, surface engineering or changing cell membrane is essential for better targeting the disease-related sites. Both chemical modification and gene synthesis are widely used techniques, but they frequently include unknown dangers or clinical application concerns. It makes sense to learn how to develop and maintain repeatable, affordable, and controllable quality. The development of improved and effective immunotherapies, such as CAR-T cells and dendritic cells-based vaccines, may provide us with a suggestion to enhance or create new modification approaches for meeting clinical requirements (Zhang et al. 2021).

Bioinspired Membrane Coated Nanoplatform for Treatment of Bacterial Infections

Nanodecoy-immunotherapy

Bacterial infection is becoming significant health concern for people worldwide and is intimately correlated with the host immune system. The bacteria are adapted to their host by dodging the immune system which contributes to antibiotic resistance. Most bacteria initially attach to the cells and tissues of the host, increasing their infectious rate and facilitating immune evasion (Palmela et al. 2018).

Therefore, exploiting the underlying mechanism of infection and coming up with new innovative ways to overcome it might aid in preventing bacterial infection. Nanotherapeutics based on cell membranes have recently demonstrated their unique benefits as anti-bacterial. One of the most serious infectious diseases, sepsis is brought on by bacteria involving systemic inflammatory reactions. If patients with sepsis are not given the right care, they may develop organ dysfunctioning or failure. Recently, red blood cell membranes-coated cores were synthesized for detoxication purposes. These nanosponges can neutralize bacterial toxins and pesticides that may cause the death in humans as well as animals (Chen et al. 2019).

These nanosponges may also considerably detoxify staphylococcal alpha-toxin, particularly in toxin-challenged mouse models increasing survival rates. Furthermore, using of the nanosponges in methicillin-resistant *Staphylococcus aureus* infection showed therapeutic efficacy. The red blood cell membrane-based nanosponges provide considerable survival benefits in MRSA-challenged mice model. Furthermore, the red blood cell nanosponges reduce lung damage and inhibit nuclear factor kappa B activation in the spleen in MRSA sub lethal dose mouse models. The findings showed that RBC-NS was a viable therapy for severe MRSA infectious diseases. Furthermore, the RBC-NS reduced lung damage and inhibited nuclear factor kappa B activation in the spleen in MRSA sub lethal dose mouse models. Furthermore, immune cells (monocytes and macrophages) can capture or trap endotoxins by binding to the membrane-inherited receptors or proteins of immune cells using the lipoplysacchrides endotoxins which are released from lytic or dead bacteria in the form of the pathogen-associated molecular pattern (PAMP) (David et al. 2020).

Neutralization of Bacterial Toxins

Pathogenic bacteria can produce toxins which cause lethal diseases. Endotoxins and exotoxins, two types of bacterial toxins, are the main culprits behind cytokine release brought on by bacterial infections. Both types of toxins are destructive and involved in several crucial pathogenic pathways. So, these must be neutralized to disarm microorganisms and directly treat infection-related symptoms (Rasko and Sperandio 2010).

Toxins must somehow interact with biological membranes depending on the cell membrane function. Cell membrane-coated nanoparticles may take advantage of high affinity receptors on several of these interactions to increase their potency. The cell membrane-coated nanoparticles have been classified on the basis of poisons against which they showed therapeutic efficacy (Fang et al. 2015).

Exotoxins

Exotoxins are chemically protein-based poisons that are so toxic that even a minute dosage may lead to organism death (Stevens et al. 2007). Gram-positive bacteria are predominantly responsible for secreting bacterial exotoxins. Traditional detoxification techniques for exotoxins depend upon the specific binding of a single antigen with an antibody. These techniques require complete knowledge of the structure of the toxin and the kind of pathogenic bacteria presenting several difficulties for clinical application. By covering the polymeric nanoparticle cores with the red blood cell membrane, a biomimetic nanoformulation system known as nano sponge was introduced to get over the therapeutic barriers (Hu et al. 2013).

The nanosponge was developed to prevent the hemolytic effect of the lethal toxin on erythrocytes. The ability of nanosponges to neutralize bacterial exotoxins was observed in vivo. The injection of alpha-hemolysin alone significantly injured mice's skin and underlying muscular tissue; however, when the toxin was combined with nanoparticles the mice's mutilation was restricted (Hu et al. 2013).

According to natural cell membranes, a monoclonal antibody plasmid against α-hemolysin was produced by genetic engineering and then transfected into cells to form an efficient antibody on the cellular membrane. The sensosenitizer Meso-tetrakis chromic was then enclosed inside these antibody membrane vesicles which were removed from the antibody modified cell membranes. The genetically produced ANVs were more effective in capturing exotoxins than passive harmful absorption through natural red blood cell membranes because of the strong interaction between the highly active antibodies and the toxin. When activated by ultrasound, the ultrasonic sensitizer nanoparticle core may efficiently produce reactive oxygen species, facilitating antibodies in removing the toxin and eliminating microorganisms. Because of the extremely particular interactions between monoclonal antibodies and the son sensosnitizer intrinsic luminous features, these monoclonal antibodies assisted nanoparticles also enabled accurate optical detection of MRSA infections (Pang et al. 2019).

The ANVs-mediated antimicrobial sonication and antitoxin therapy were shown to entirely eradicate muscle abscesses in mice when used in combination, according to in vivo research (Guerrant et al. 1999). The antitoxin treatment approaches do not target specific bacteria and prevent the natural selection of antibiotics against drug-resistant bacteria. So, these are a viable alternative to conventional antimicrobial therapy (Mellbye and Schuster 2011).

Endotoxins

Endotoxins also known as lipopolysaccharides (LPS) are components of gram-negative bacteria's cell walls produced by the inner membrane upon death, division or after receiving antibiotic drugs (Shen et al. 2019). Immune cells including neutrophils, dendritic cells, and macrophages can identify these endotoxins as pathogen-associated molecular patterns (Akira et al. 2001).

The systemic inflammatory response syndrome which includes sepsis, multi-organ failure, shock and intravascular coagulation, can be brought on by the increasing activity of the mammalian immune system caused by lipopolysaccharides (Yuk et al. 2021). Therefore, the important treating disorders is the efficient elimination of LPS. LPS can be trapped by macrophage membrane-coated nanoparticles which can also suppress the immune response. Macrophage membrane TLR-4 and CD-4 receptors are linked to LPS binding and the release of downstream inflammatory components. Utilized the LPS-binding properties of macrophage membrane-coated PLGA nanoparticles to reduce the level of pro-inflammatory factors (Thamphiwatana et al. 2017).

Vaccine-immunotherapy

A crucial defense against bacterial illnesses is vaccination. Due to the distinctive properties of nanoparticles and cell membranes certain cell membrane coated nanoparticle oriented vaccines have been investigated to prevent several lethal infections. Generally, nanoparticles are readily absorbed by cells enabling the antigen associated with nanoparticles to be rapidly ingested and processed by antigen-presenting cells. The antigen bridging on the nanoparticle surface perfectly mirror the natural appearance and presentation of pathogenic antigens. Nanoparticles and plasma membranes can be made with various infectious pathogens to serve as vaccine delivery systems.

Based on their antimicrobial action these antimicrobial vaccines can be divided into two primary categories: antibacterial vaccines and antitoxin vaccinations (Angsantikul et al. 2018).

Antibacterial Vaccines

Inactivated bacteria, attenuated bacteria, bacterial component antigens, or inactivated bacterial toxins make up the majority of traditional antibacterial vaccinations. Each year, these vaccines successfully prevent millions of deaths worldwide (Plotkin 2003).

The live attenuated vaccines have high immunogenicity, but they also come with safety hazards, especially for people with impaired immune systems. Compared to this inactivated bacterium are less immunogenic than living bacteria, which means that they are less effective at producing the immune system's defence against infection. Proteins and polysaccharides present in bacterial outer membrane vesicles have the same bacterial natural immunostimulatory effects (Gerritzen et al. 2017).

Some of these vesicles have been used in clinical bacteria pathogen protection such as a meningococcal vaccination where it has been shown to boost the host immune response (Holst et al. 2013). In particular, the outer membranes of bacteria are promising antigen-derived materials because they have enriched immunogenicity and distinctive adjuvant qualities that can activate innate immunity and trigger adaptive immune responses (Irene et al. 2019).

The outer membranes of bacteria were gathered and coated onto gold nanoparticles to create an antibacterial vaccination (Gao and Zhang 2015). The study involved the extraction of *E. coli* outer membrane vesicles and their unique wrapping onto tiny gold nanoparticles of diameter 30 -50 nm. This is due to the gold nanoparticles (AuNPs) distinctive size and form, which would be specifically tuned to increase the rate of an immune response. In addition to their high stability, the subsequent subcutaneously administered bacterial membrane-coated AuNPs may quickly activate and mature dendritic cells in vivo. Additionally, the BM-AuNPs stimulate the increased production of IFN-g and IL-17 in vivo but not IFN-g or IL-4, indicating that they can specifically target and induce Th1 and Th17 preferred cell responses to destroy the pathogenic bacteria. The research demonstrated a significant promise for antibacterial vaccinations by using bacterial outer membranes to surround synthetic inner cores. Similar to earlier research, bacteria-related antigens integrated into cell membrane-coated nanoparticles were utilized as a vaccine to prevent lethal infection by stimulating the immune system of the body against pathogen-associated antigens. Recently, multiple cytolysins or proteins released by gram-negative bacteria attached to the macrophage membrane and were covered on PLGA nanoparticle as a multiantigen nanotoxoid vaccine to strengthen the immunity against bacteria resistant to antibiotics (Wei et al. 2019).

Antitoxin Vaccine

Vaccines which are effective against toxins, are usually made from toxoids that have undergone chemical and thermal treatment for safety reasons still these inactivation techniques can degrade proteins tertiary structure, reduce immunogenicity and alter antigenic epitopes (Qin and Sun 2020).

The characteristics of cell membrane coated nanoparticles such as antigen distribution over membrane and toxin adsorption, are considered helpful in creating antitoxin vaccines. A new method for creating multiantigen vaccines utilized red blood cell-coated nanoparticles that can absorb toxins to generate high immunogenicity without adjuvants (Wei et al. 2017).

Chemo-immunotherapy

Antibiotics have been extensively developed and used in modern medicine, but still a big challenge of active targeting of antibacterial drugs particularly for bacterial infections. Antibiotics are frequently given systemically to ensure that they kill or at least inhibit the growth of harmful bacteria, however doing so increases the risk of antibiotic resistance and toxicity. The synthesis of CMCNPs that may actively target diseased microenvironments and pathogen may provide an efficient therapeutic approach against microbial infections in recent decades (Lobanovska and Pilla 2017).

The bacteria has the tendency to attach themselves to the surface of platelets and then produce several virulent toxins that ultimately damage the platelets and increase the severity of bacterial infection (Theodora et al. 2018). One of these bacteria is MRSA, which potentially releases α-toxin leading to the aggression of platelet and inflammation, making the pathogen too strong to be cleared by the immune system. Keeping the pathogenic mechanism in mind, scientists successfully developed platelet membrane-coated therapeutics. The platelet membrane was shawled onto the nanoparticles loaded with vancomycin and administered to target MRSA (Hu et al. 2015). Platelet membrane-coated nanocores evaluated to have greater binding power with MRSA than the bare nanoparticles or red blood cell membrane-wrapped nanoparticles. Moreover, vancomycin-loaded platelet membrane coated nanoparticles showed more potent bacterial inhibition than bare vancomycin-loaded nanoparticles. Ultimately, the bio interfacing antibacterial strategy enhance antibiotic potency (Yesi et al. 2022).

Additionally, the bacteria interact with platelets, such as direct bridging with surface proteins or connecting with plasma molecules, among other mechanisms. For instance, by adhering to platelet membranes, MRSA can evade the immune system's clearance. Researchers discovered that vancomycin-loaded platelet membrane-coated nanoparticles (PNPs) showed a 12 times higher capability to bind with MRSA than bare nanoparticles. In a mouse model of MRSA attack, this potent combination significantly increased the bactericidal efficiency and was six- folds more effective than a free antibiotic (vancomycin) (Hu et al. 2015).

Sono-immunotherapy

Sono-immunotherapy may be a viable option for treating bacterial infections. Antibiotic-free protocols may provide a promising role in preventing and controlling resistance against bacterial infection through eradicating of pathogenic bacteria and pathogenic virulence. A bioinspired method was considered for combining antibacterial sonodynamic therapy with the antivirulence immunotherapy. The technique involved genetic engineering of the surface of plasma membrane nanovesicles via an antibody which aids in neutralizing the toxin of methicillin-resistant *Staphylococcus aureus* (MRSA) through son sensitizer encapsulation. Compared with the old conventional passive virulence absorption technique, the sono-immunotherapy provides strong junction between antibody and alpha-toxin enabling the nanovesicles to absorb pathogenic virulence more effectively in the lab. The underlying principle is that, the sonosensitizers effectively produce reactive oxygen species to kill the pathogen and enhance the clearance of virulence. In vivo testing through optical imaging indicated that the antibody decorated nanovesicles successfully targeted MRSA infection and accurately identified the foci from sterile inflammation. So, these antibiotic decorated nanocaptures may robustly fight against resistant bacterial infections (Pang et al. 2019). Fig. 1 and Table 2 summarizes the different therapeutic approaches of biomimetic nanoparticles for treating the bacterial infections.

Perspectives and Challenges

The membrane components, including membrane polysaccharides, proteins and lipids have been moved to the surface of the nanoparticles. CMCNPs naturally possess the functions and characteristics of derived cells. By taking advantage of interactions between pathogenic bacteria or the contaminated microenvironment and the cell membrane coating, these CMCNPs can be employed for various therapeutic objectives. This approach is adaptable and permits the fusion of various nanoparticles and plasma membranes depending on the therapeutic goal. Additionally, genetically modified plasma membranes of various cells can be employed to provide capabilities that donor cells lack (Ren et al. 2021).

Using these methods as inspiration it will be possible to express many antimicrobial components on cell membranes at once to create a multifunctional cell membrane that can treat bacterial infections. For example, antimicrobial peptides might be genetically designed on cell membranes to kill bacteria. The pathogen recognition receptors might be expressed on plasma membranes to improve the capacity of the membranes to target specific pathogenic bacteria. However, some bacterial species remain in the host for a long time and have a higher ability for attaching to the host cells to maintain their growth and pathogenic activity leading to long-lasting and chronic infections. Extracting cell membranes from host cells via in vitro culture could decrease inflammatory reactions and provide more precisely targeted antimicrobial medication. Such host-derived cell membranes have lower immunogenicity and are better able to target pathogenic germs (Yu et al. 2021).

Conclusion

One of the major threats to human life comes from bacterial infections, and traditional antimicrobial strategies such as vaccination, antibiotic resistance, and drug delivery are ineffective. Due to the use of NPs and cell membranes, cell membrane coated nanoparticles (CMCNPs) have a distinct advantage in treating of bacterial infections. It is anticipated that CMCNPs would be developed into a very effective antibacterial and antitoxin vaccine. It can give more targeted delivery of antibacterial medications and efficiently eliminate the bacterial toxins. CMCNPs can offer a new

Table 2: Cell membrane coated nanoparticles used for the treatment of bacterial infections

Types of Cell Membrane	Methods of preparation	Characteristics of Membrane	Material used for Nanoparticles	Antibacterial Mechanism of action	Limitations	References
Red Blood Cell Membrane	Repeated freezing and thawing under isotonic conditions	Long-term systemic circulation of toxins and immune evasion	Gold PLGA	Antitoxin vaccination that neutralizes exotoxins over a broad spectrum	Prolong storage and lack of cell sources	Gao et al. 2015: Hu et al. 2013
Neutrophil Membrane	Sonication	Enlarge at inflammatory sites and have a prolonged pulmonary circulation	Ceftazidime, RvD1, and PCL pefloxacin and PEG	Targeting the microenvironment of microbe's infection	Limitations of cell sources and long storage	Gao et al. 2021: Wang et al. 2020
Bacterial vesicles with a cell wall	Ultrafiltration and ultra-high speed centrifugation	Homologous, natural immunostimulatory targeting	Mesoporous silica, rifampicin, and gold	Medication delivery and vaccination against microorganisms	low density of cell membranes, immunogenicity	Gao et al. 2015: Wu et al. 2021
Platelet Membrane	Isotonic therapy and repeated process of freeze and thaw	Specific bacterial binding protein and cell signaling	PLGA,	Emphasis on harmful microorganisms	Absence of cell sources and prolonged storage	Hu et al. 2015
Dendritic Membrane	Sonication	Immune escape, CD14, and strain receptors are present on the surface	CuFeSe2	Destroy Endotoxins while emphasizing on pathogenic microbes	Describe pro-inflammatory factors	Hou et al. 2021
Macrophase Membrane	Sonication	Immune evade, CD14, and toll-like receptors are expressed on the surface	Gold nanocage, PLGA, and $Fe_{3}0_{4}$	Eliminate endotoxins while relying on harmful bacteria	Discuss pro-inflammatory substances	Thamphiwatana et al. 2017: Wang et al. 2018: Shen et al. 2018
Genetically Engineered Membrane	Sonication	Elevated antigen and antibody sensitivities	Sonosensitizer	emphasizing on bacterial infections, neutralizing exotoxin, and diagnosing infection with pathogenic bacterial exotoxin	Extreme technical demand	Pang et al. 2019
Hybridoma Membrane	Depend upon the type of cell membrane used	Integrate many cells membrane processes	PLGA	Eliminate both endo and exotoxins, concentrating on harmful microorganisms	Control of hybrid mitogenic quality	Dehaini et al. 2017: Jiang et al. 2021
Gastric Epithelial Cell Membrane	Sonication	An assortment of receptors that are developed bind to Helicobacter pylori	Clarithromycin and PLGA	Emphasis on pathogenic organisms	Inadequate cell sources	Angsantikul et al. 2018

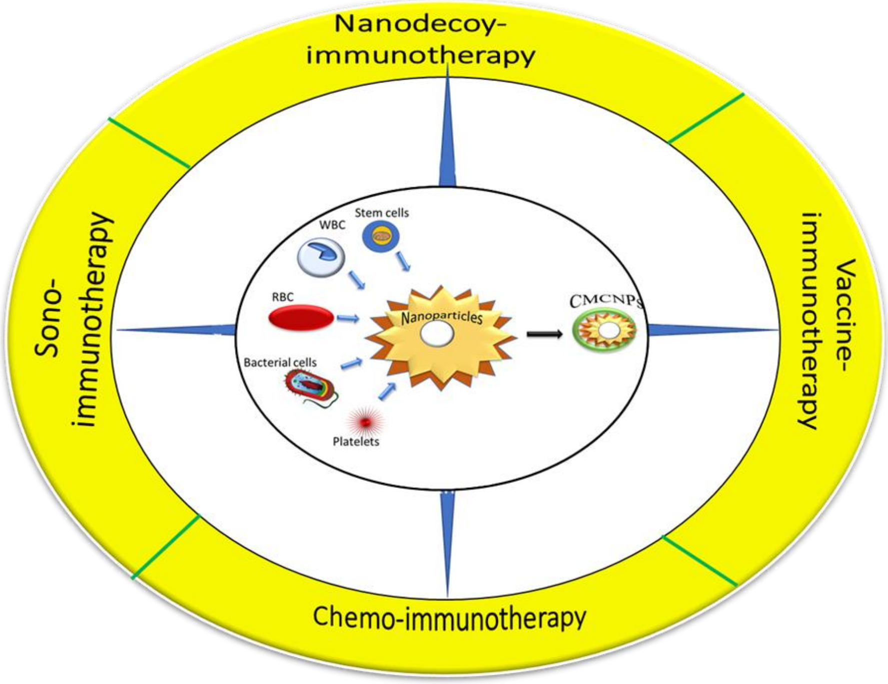

Fig. 1: Antibacterial therapeutic strategies of cell membrane coated nanoparticles

approach to successfully reduce bacterial drug resistance when combined with photodynamic and sonodynamic therapy. Even though a large portion of the research is still in the laboratory, future developments in this area are anticipated to benefit human health.

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Clostridium difficile Infection (CDI) in Human-Animal Interface: A One Health Perspective

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INTRODUCTION

A complex flux of microbes colonizes our gastrointestinal tract (GIT) from the day we are born. The balance of the symbiotic relationship between the intestinal epithelium and the bacterial, fungal, and parasitic microflora is pivotal for good health at any point in the life. Oral contamination of food particles, aided by broncho mucociliary transportation, is digested and sterilized in the stomach's acidic environment. Incursion, beyond this stage is dependent on stomach pH and microbes' capability to transit as resilient spores or stay concealed in mucus membranes. Passage through the small intestine causes a lumen multiplication of around 10^6 bacteria per milliliter reaching the colon, where aqueous resorption raises the concentration to 10^{12} bacteria per gram of fecal matter. Because of the luminal gap between the mucosal oxygenic vessels, the microenvironment favors a thousand to ten thousand fold for most of the anaerobes (Novogrudsky and Plaut 2003).

Clostridium and Bacteroides-Prevotella account for roughly 99 % of all anaerobic bacteria found in the colon (Mai and Morris 2004). Throughout all ages, gut homeostasis relies upon non-motile and enterocytic bacterial interactions, involving cellular adhesion and immunological regulation. The term "colonization resistance" is often used to describe this defensive condition of the natural intestinal ecology (Britton and Young 2014; Murray et al. 2020). Pathogens such as *Clostridium (C.) botulinum*, *C. perfringens* and *C. tetani* produce potent toxins that cause devastating human diseases, including food poisoning, gas gangrene, and tetanus (Khan et al., 2021). *C. difficile* is a prominent enteric pathogen that causes colitis and toxin-mediated diarrhoea, known as *C. difficile*-associated diarrhoea (CDAD) or *C. difficile* infection (CDI). CDI has traditionally been regarded as a hospital-associated disease (Feuerstadt et al. 2023). Even though the earliest outbreaks of *C. difficile*-associated diarrhoea were linked to clindamycin usage, it wasn't until the advent and extensive utilization of broad-spectrum 3rd generation cephalosporins, a class of antibiotic to which *C. difficile* is innately resistant (Slimings and Riley 2014). Pulsed-field gel electrophoresis type NAP1/ restriction endonuclease analysis group BI/PCR ribotype (RT) 027, a "hypervirulent" fluoroquinolone-resistant strain of *C. difficile*, was identified as being North American in origin based on the typing method (He et al. 2013), causing a sharp 4-fold rise in CDI prevalence in Canada and a tripling of fatality rates. The global prevalence of CDI keeps rising in several high income per capita nations (Slimings et al. 2014; Lessa et al. 2015).

Furthermore, a 'novel' characteristic of CDI has emerged. Formerly infrequent community-associated CDI (CA-CDI), described as symptoms beginning in the public and no prior hospitalization in the preceding twelve weeks or symptom onset within 48 hours of hospitalization, has surfaced as a significant percentage of all CDI infections. In the United States, CA-CDI accounts for 41% of all CDI incidence, a 4-fold rise over the last two decades (Khanna et al. 2012). For instance, in 2011, CA accounted for one-third of all the CDI cases in Australia (Slimings et al. 2014), with a rising number of instances over time, from approximately 12% in 2010 to 27% in 2014 (Worth et al. 2016). Interestingly, CA-CDI cases often lack the usual CDI risk factors. They are predominantly young, female, and healthy, with no prior interactions with healthcare facilities or hospitalized individuals and frequently with no record of antibiotic usage (Kuntz et al. 2011). *C. difficile* prefers to thrive in the GIT of mammals (including humans and animals). The bacteria most likely colonize the GIT of all neonate animals, multiply, and are excreted to begin the cycle again by infecting another newborn. As the young One grows older and begins to take a grownup diet, multiple microbes colonized the GIT and the predominance of *C. difficile* declines as other species outdo it (Moono et al. 2016). The use of antibiotics, particularly those that have an action against commensal gut flora, develops an environment that imitates the GIT of infants, which favors *C. difficile* proliferation. Since the 1960s, antibiotics have been administered and used in meat-

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producing animals as growth promoters (AGPs) and as a dubious disease prevention tool. (Ronquillo and Hernandez 2017) and consequently, food animals become a significant reservoir and a host for amplification for *C. difficile* (Diaz et al. 2018; Knight and Riley 2019). After 2010, a sharp increase in *C. difficile* contaminates food and the environment (Diaz et al. 2018; Knight and Riley 2019). Recent developments in WGS technology have revealed that most *C. difficile* strains found in humans, animals, food, and the environment are genetically similar and in certain instances, identical. This raises the possibility of zoonotic transmission between people and animals using contaminated food and the environment as a route (Knight et al. 2017; Janezic et al. 2018).

Virulence Factors and Toxico-kinetics of *C. difficile*

There are many virulence factors of *C. difficile* such as its potent toxins, surface-layer protein, spores, and adherence. The two main enterotoxins of *C. difficile's*, tcdA and tcdB (Rupnik et al. 2009), are responsible for its virulence, described by the breakdown of the enterocytic actin-skeleton caused by the Rho-metabolic process. Toxins A and B are prototypical members of the large clostridial cytotoxins (LCT) family and bind to an unidentified receptor on the intestinal mucosa via the carboxy-terminal end. This essential adherence might be dependent on capsular and cell-wall components like S-layer adhesions (P36 and P47) or other immunostimulatory flagellar or fibronectin-binding proteins that have been postulated (Pechine et al. 2007). Through the relocation of the center region, the toxin amino terminal is subjected to internal glucosylation of the Rho proteins required for epithelial cell cytoskeleton formation (Janoir 2016). This causes actin filaments to depolymerize, resulting in the loss of their interior structure, rounding of cells, and breakage of adherent's junctions that keep cells intact. The Inflammatory response and mucosal epithelium loss aided the deterioration of brush border membranes and the villus accelerates the epithelium's malfunctioning. The main fibrin layers arrange to cover ulceration, which stands out on inflamed necrosis debris, creating a knob-like coalescent pseudomembrane colitis (PMC) visible by endoscopy. Although strains that produce only toxin tcdB and lack a functioning toxin tcdA gene can occasionally induce toxin-mediated diarrhea. However, most clinical isolates contain both toxin A and toxin B genes, leading to synergistic pathological characteristics (Rupnik 2006).

Additionally, certain *C. difficile* strains synthesize a third type of toxin located chromosomally, known as a binary toxin (CDT). This toxin is an actin-specific ADP-ribosyltransferase with a high degree of structural and functional similarity with the ι-toxin from *C. perfringens*. Isolate that is positive for toxins A and B also produced CDT (6-30 % of strains) (Rupnik et al. 2009).

Colonization of Pathogen and Associated Diarrhoea

Antibiotic medication often alters the natural gut microbes, which is the major risk factor for contracting infections with *C. difficile* and *C. difficile*-associated diarrhea (CDAD). Various experimental animal models have shown the involvement of antibiotics therapy in dismantling colonization resistance and CDAD, whereby following the inoculation of normal fecal flora transiently eliminates *C. difficile* and symptoms. The relevance of colonization resistance repair is supported by both in vitro inhibition of *C. difficile* growth while subjected to fecal emulsions from healthy individuals and in vivo, the use of curative nasogastric replenishment of normal intestinal flora in human CDAD (Aas et al. 2003). Asymptomatic transmission of *C. difficile* in healthy individuals in the community is very rare (1-15%), raising concerns about the endogenous origin of *C. difficile* in CDAD. However, current findings have shown that in more than 50% of healthy individuals, a toxin tcdB is present (Iizuka et al. 2004). This suggests that *C. difficile* population is present in less proportion rather than nonexistent in healthy intestinal microflora. On the contrary, the environment could potentially serve as a constant source of infection that is amplified by factors, for instance, medical wards that are highly contaminated. *C. difficile* carriage, either intermittent or persistent, tends to lead to CDAD. This is common whenever patients are hospitalized since 15-21% of individuals are colonized with *C. difficile* colonization and antibiotic therapy stimulates the beginning of CDAD (Kyne et al. 2000). *C. difficile* is spread via infected persons, ambient objects, and medical care staff's hands. While asymptomatic carriers won't experience diarrhea (Bobulsky et al. 2008) and they will help the spores spread throughout the environment (Riggs et al. 2007). Although the colonization of *C. difficile* is the primary risk factor, which is often acquired after hospitalization, there are many other significant associated risk factors, which are highlighted in the diagram (Fig. 1). The use of antibiotics is the most significant risk factor and practically all antibiotics currently available on the market have been associated with CDAD (Johnston et al. 2012).

Cephalosporin, Clindamycin, and Ampicillin are the most frequently mentioned drugs accounting for almost 90% of all precipitating antibiotics in CDAD (Leffler and Lamont 2015). Unsurprisingly, a prolonged antibiotic regimen or multiple antibiotic usages raises the chance of CDAD. Antibiotics with minimal risk, such as tetracyclines, rifampicin, aminoglycosides, trimethoprim and the older generation of quinolones, have little effect on the normal inhabitant anaerobes and are rarely the reason for CDAD. Since the advent of newer generations of quinolones class with enhanced anaerobic activity, there is an increased risk of CDAD after using moxifloxacin, gatifloxacin (Gaynes et al. 2004) and levofloxacin (Khurana et al. 2004). This is further shown by the widespread outbreaks in the United States and

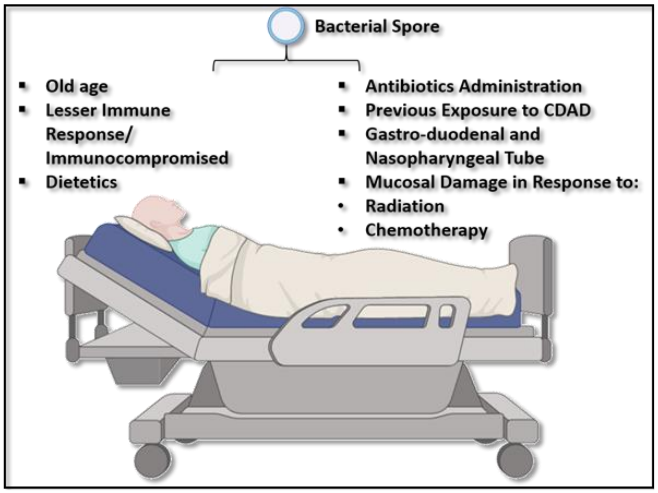
Bacterial Spore
Old age
Lesser Immune Response/ Immunocompromised
Dietetics
Antibiotics Administration
Previous Exposure to CDAD
Gastro-duodenal and Nasopharyngeal Tube
Mucosal Damage in Response to:
Radiation
Chemotherapy

Fig. 1: Possible associated risk factors of *C. difficile*-associated diarrhea (CDAD)

Canada (Loo et al. 2005) caused by increased fluoroquinolone prescription. Effective strategies such as limiting the usage of cephalosporins lower the prevalence of CDAD. In older people ages sixty to ninety, the risk of CDAD increases ten times, as well as other known risks of the host, such as compromised immunity and decreased levels of antibodies against toxin tcdA and tcdB, might further complicate matters (Leffler and Lamont 2015).

Furthermore, a concurrent disease that involves regular hospitalization or an extended stay in the hospital, including many precipitating therapies, such as antineoplastic/ chemo therapy and endotracheal feeding tubes, will result in persistent CDAD (Hung et al. 2013). Using newer proton pump inhibitors (PPI) or laxatives has been linked to an increased risk of CDAD to some extent (Dial et al. 2004). It has been suggested that dietary risk factors for CDAD include starvation after surgery or a diet lacking in non-essential amino acids like proline and cysteine. However, these conditions must be thoroughly investigated in actual clinical settings (Johnston et al. 2012).

Clinical Manifestation of CDAD

CDAD is triggered by alteration in the intestinal flora, a high proportion of toxin-producing *C. difficile*, nutritional imbalance, a compromised host immune system and other variables that regulate toxin release. The interaction of these factors influences the onset of diarrhea, which is commonly defined as a minimum of three loose faeces/ day and represents a self-limiting sickness. CDI's clinical manifestations are quite diverse and may vary from the asymptomatic carrier status, mild to severe diarrhoea, to a life-threatening condition, fulminant colitis. The incubation period is not well defined and some publications imply that it lasts for two to three days. The most recent research has shown that it may last much prolonged than that and varies from person to person (McDonald et al. 2018). CDI may damage any section of the colon, but it is typically seen in the proximal portion. The majority of patients infected with CDI have moderate diarrhea and recover on their own after a week of antibiotic withdrawal. Diarrhea develops in the majority of cases either during or immediately post-antibiotic medication, while CDI may develop days later. Along with watery diarrhea, CDI's clinical manifestations include pyrexia, nausea, vomiting, fatigue, and anorexia. Despite the rarity of actual bleeding, stool testing for blood are frequently positive (McDonald et al. 2018). The signs and symptoms of extreme clinical manifestation of CDI are all potentially fatal such as severe dehydration, abdominal distention, hypo-albuminemia with edema and eventually vascular shock. Extremely sick individuals may seem to be improving clinically by resolving diarrhoea; however, this is typically a symptom of ileus with the appearance of toxic megacolon. Aside from the signs mentioned before, other serious CDI adverse effects include paralysis of the intestine, colonic perforations, renal failure, systemic inflammatory response syndrome (SIRS), septicemia, and ultimately death (McDonald et al. 2018). Despite life-saving surgical treatment, oral medication has less impact at this point, and the death rate has increased to 25–40%. Outside the colon, the clinical manifestation of CDI are uncommon and often include small intestine invasion, reactive arthritis, and bacteremia. Because severe concurrent illnesses might complicate the assessments, it can be challenging to estimate the mortality rate in CDAD (Dendukuri et al. 2005).

Mortality related to CDI complications is about 15–25% and may reach up to 34% in critical care units (CCU); while on the other hand mortality rate directly linked with CDI is estimated to be 5%. When comparing CCU patients with and without CDI, mortality rates increase twofold (Sidler et al. 2014; Czepiel et al. 2015). The poor prognosis is linked to old age, high leukocytosis, hypo-albuminemia, and elevated levels of creatinine (Vaishnavi 2010). Moreover, it has been demonstrated that the first CDI clinical episode raises the overall probability of fatality. Reoccurrence of signs and symptoms of CDI usually appears within the first week following the initial infection, after therapy is completed. Following successful antibiotic therapy of the initial CDI illness, at least one subsequent recurrent disease happens in 10-25% of patients and also occurs in more than 65% of affected patients who have undergone more than one recurrence of CDI (McFarland et al. 1999). According to data, 50% of recurring infections are caused by relapsing of the primary strain, while the remaining 50% are caused by re-infection with other strains. Recurrences are considered to be caused by a compromised host immune system to A and B toxins, along with exposure to spores. Drug resistance seems to have little effect on the likelihood of recurrence (Vaishnavi 2010; Moore 2018).

Diagnosis

Whenever diarrhea symptoms appear (loose faeces more than three times/ day), CDI must be considered immediately. CDI is diagnosed by detecting *C. difficile* toxins (tcdA, tcdB) directly from a faecal sample, most often using an enzyme immunoassay (EIA) that offers a quick turnaround (less than 2 hours) with a sensitivity of 75-85% and specificity of almost 95-100%. It is one of the most widely used assay used in laboratory settings because it is less expensive and easy to use. Antigenic tests for diagnosing *C. difficile* is found mainly on detecting glutamate dehydrogenase (GDH). They are distinguished by their convenience of use, quick response time, and high specificity of about 100%. The only drawback is the low specificity (59%). It fails to differentiate between the strain's toxigenicity and non-toxicity (Bartlett 2010; Simor 2010). In 2009 nucleic acid amplification test (NAAT) was introduced. These tests use the amplification of nucleic acid based on either PCR technique or isothermal amplification. Compared to EIA, NAAT has higher sensitivity and specificity.in case of a negative result, the specificity increases significantly and reaches 95% (Su et al. 2013; McDonald et al. 2018). The NAAT also has certain disadvantages, including significant expense and result in interpretation challenges. The expression of a toxin-encoding gene is confirmed by PCR, which also verifies the existence of a toxigenic strain of *C. difficile*. However, this doesn't certainly imply that the strain is currently synthesizing any toxins. If the diarrhoea is caused by anything else, detecting this strain would be deceptive since it results in additional medication for CDI. Cytotoxic assay tests (CYTA) are not often employed in microbial cultivation because these are laborious and time-consuming with response time of 48 to 72 hours (Leffler and Lamont 2015). The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) recommends no test is available solely for the confirmation of CDI. Combining two assays in an algorithmic approach is the most effective technique for the optimization of CDI diagnosis. The initial test needs to be one with a higher negative predictive accuracy (NAAT or GDH) and the following test should have a higher positive predictive value (EIA). If the initial test result is negative, CDI is ruled out. EIAs should be carried out if the results are positive. CDI is confirmed when the subsequent test results are positive (McDonald et al. 2018). Fig. 2 illustrates the schematic flow sheet for CDI diagnosis.

But if the second test comes out negative, the situation must be reviewed clinically. This may be seen in 3 scenarios: (1) a low level of toxin (below the threshold limit), remains undetected, (2) a false-negative result of EIA (3) carrier of *C. difficile*. Retesting is necessary for samples showing a negative result for GDH but positive for toxin since it's an invalid result (Crobach et al. 2016).

CDI in Animals Interface a Potential One-Health Threat

One Health is a collaborative approach emphasizing that our health and well-being are inextricably linked to animal health and the environment. A key aspect of the One Health initiative, generally and especially for CDI prevention, expanded the collaboration between doctors, veterinarians, farmers, and legislators to improve surveillance and monitoring, research, and establish integrated national policies. For a long time, doctors and vets used non-divergent approaches to treat CDI (Gerding et al. 2008). Public health workers still consider CDI to be solely a hospital-acquired problem and are usually ignorant of the frequency of toxin-producing *C. difficile* in our environment, its One Health implications, such as the fact that *C. difficile*-related death in newborn pigs may exceed 50%, with huge economic ramifications for industries. (Songer and Uzal 2005). Despite previously thought to be a hospital-acquired illness spread from One infected individual to another, Eyre et al. (2013) conducted a study to investigate the intrinsic genetic epidemiology of CDI. The researchers employed highly sophisticated techniques like WGS and core-genome single nucleotide variant (cgSNV) analysis. They analyzed *C. difficile* isolates from 957 hospital- and community-based CDI patients that have been gathered during 2008 and 2011 from UK and discovered that just 35% of isolates were genomically connected to at least one prior case while 45% of isolates were genomically unique from all preceding cases. About 126 out of 333 isolates with an indication of clonal transmission had frequent hospital interaction with some other patient, and 120 isolates had no conceivable epidemiologic relationship with another patient in the healthcare setting or the community. It was proposed that, in contrast to infected patients, other environmental reservoirs potentially have a significant unreported involvement in *C. difficile* dissemination.

According to recent investigations by Sheth et al. (2019) and Halstead et al. (2019), compelling evidence for asymptomatic patients have an important role in CDI transmission in healthcare facilities. In these investigations, asymptomatic individuals were tested for *C. difficile* upon hospitalization, and the resultant isolates were subsequently subjected to WGS and contrasted with isolates from symptomatic CDI individuals using other available techniques like MLVA or cgSNV analysis. About 10-15% of those tested subjects were asymptomatic *C. difficile* carriers, with >80% colonized by toxin-producing strains that can cause illness. These investigations provided evidence for the hypothesis that asymptomatic carriers were spreading *C. difficile* to vulnerable hospitalized patients by transferring the infection from asymptomatically infected persons to patients that are previously negative for *C. difficile* and by grouping asymptomatic individuals with symptomatic CDI patients. Likewise, further different research by Gonzalez-Orta and

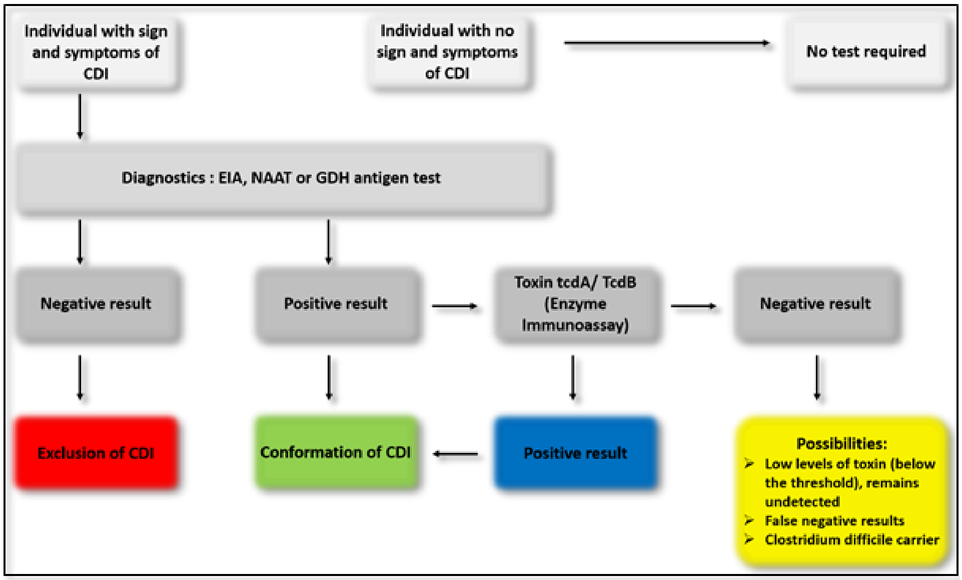
Individual with sign and symptoms of CDI
Individual with no sign and symptoms of CDI
No test required
Diagnostics : EIA, NAAT or GDH antigen test
Negative result
Positive result
Toxin tcdA/ TcdB (Enzyme Immunoassay)
Negative result
Exclusion of CDI
Conformation of CDI
Positive result
Possibilities:
Low levels of toxin (below the threshold), remains undetected
False negative results
Clostridium difficile carrier

Fig. 2: Flow sheet for the diagnosis of CDI.

associates in the USA demonstrated that nearly 25% of hospital-related CDI cases were diagnosed with strains that the patients had already been colonized at the time of hospitalization (Jencson et al. 2019). This implies that there weren't healthcare facility cases and CDI acquired in the community, with the illness only after hospitalization. With the continuing importation of *C. difficile* strains into the healthcare setting through asymptomatic carriers, community sources/reservoirs may be having a significantly larger role in the transmission of CDI than previously assumed, and the frequency of CA-CDI may have been substantially misrepresented underneath the established categorization rules. Up till now, *C. difficile* is isolated from a wide range of reservoirs/sources such as human-animal (Food animals, seafood, and meat) and environment (natural and household environment) interface (Knight and Riley 2019). The environment (natural and household) and consumable foodstuff (retail food and food animals) are all significant *C. difficile* reservoirs. The infection rates in the newborn are typically high, up to 70% in piglets, and vary between 15-20% in calves. A higher prevalence (42%) of *C. difficile* was reported in the United States (DePestel and Aronoff 2013). On the other hand, findings from a European study, point out a far lesser number (only 3%), maybe because of different slaughtering management practices (Candel-Perez et al. 2019). *C. difficile* is also often found in natural settings including water and soil where its incidence ranges from 30 to 50 %. In these investigations, the most prevalent strain was *C. difficile* RT 014 belongs to MLST cluster 1 (ST2, ST13, and ST49) and cluster 5 (ST11 RTs 33, 78, 126, and 127). In humans, CDI is associated with the lineage RT 014 and ST11 (Knight and Riley 2019). Both possess wide genomes and consistently evade the effects of many antimicrobials being used in human and veterinary practices (Knight et al. 2017; Knight et al. 2019). Thus, this again emphasizes how *C. difficile* is relevant to the concept of One Health, which states that three separate issues need an integrated solution i.e., human-animal health and the environment. Currently, no concrete evidence exists that *C. difficile* is transmitted via faeco-oral or the environmental route. This evidence is hard to get because (1) not every person exposed to *C. difficile* exhibits signs and symptoms (depending on the susceptibility of their gut microbiota), (2) *C. difficile* is widespread, and (3) the sporulation capability of *C. difficile* (spores can stay quiescent till the favourable environment for the propagation). Furthermore, the infectious dosage (ID_{50}) for CDI in the human population is uncertain. Nonetheless, thanks to the advancement in microbial genetics, presently there is sufficient and persuasive proof that *C. difficile* endemic to humans and farm animals has a contemporary evolutionary history, and also that CDI has a significant zoonotic element, resulting in contamination of consumer food and the environment. Based on an investigation by Knetsch et al. (2018), the results of core genome analysis showed considerable clustering of human and animal strains, indicating that *C. difficile* may transmit both ways between farm animals and humans. A clonal cluster RT 078 revealed transcontinental transfer between an animal in Canada and humans in the United Kingdom. Tetracycline is extensively employed in veterinary medicine. In a study by Dingle KE et al. (2019), tetracycline preference was recognized as an essential driving factor for the evolution of *C. difficile* RT 078, with various tetM-related clonal expansions happening in early 2000. An agrarian perspective for *C. difficile* RT 078 is even further supported by the emergence of various tetM components, which are Tn916-like components from proven zoonotic pathogens such as *E. coli*, *E. faecalis*, and *S. suis*. In Australia, *C.*

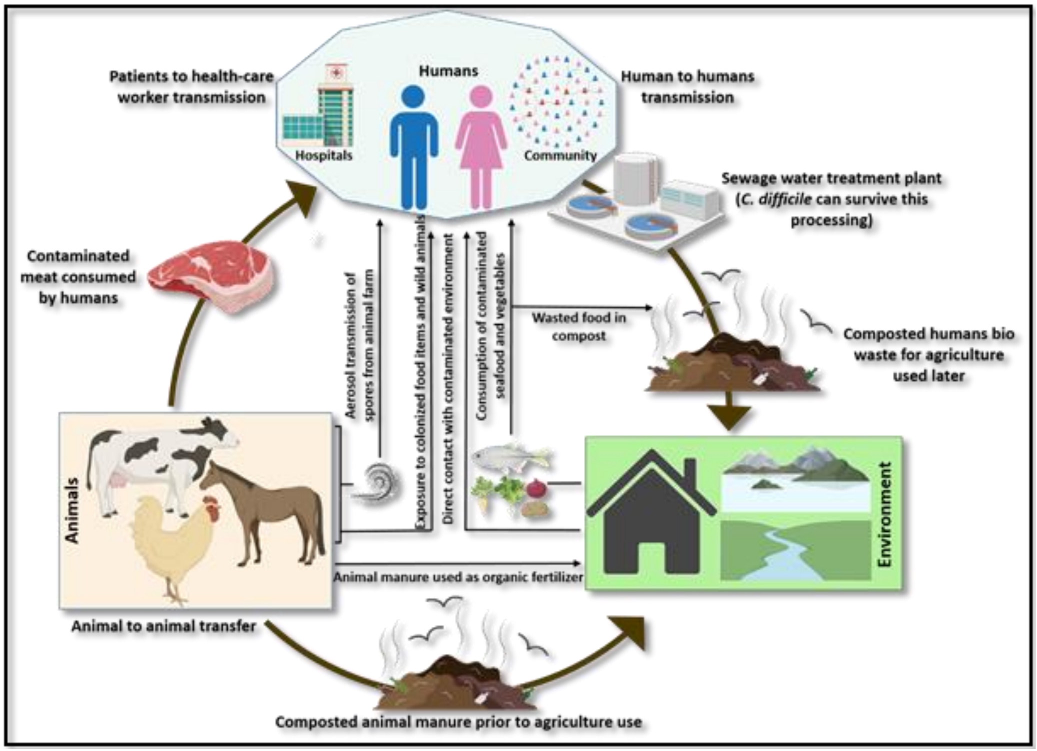
Patients to health-care worker transmission
Humans
Human to humans transmission
Hospitals
Community
Sewage water treatment plant (C. difficile can survive this processing)
Contaminated meat consumed by humans
Aerosol transmission of spores from animal farm
Exposure to colonized food items and wild animals
Direct contact with contaminated environment
Consumption of contaminated seafood and vegetables
Wasted food in compost
Composted humans bio waste for agriculture used later
Animals
Environment
Animal manure used as organic fertilizer
Animal to animal transfer
Composted animal manure prior to agriculture use

Fig. 3: Transmission cycle of *Clostridium difficile.*

difficile RT 014 is very common in humans and pigs with CDI, accounting for about 30% and 25% of isolates, respectively (Knight et al. 2015; Collins et al. 2017). A researcher (Knight et al. 2017) isolated and sequenced the collection of the RT 014 strain of *C. difficile* from humans and pigs at the same time. The results of the cgSNV analysis showed current cross-species transmission, with 42% of strains isolated from humans sharing a clonal connection with at least one animal isolate. Furthermore, it is doubtful that there's any direct interaction between both humans and animals since these clones were isolated hundreds of miles away throughout several Australian states. By considering all the facts it seems that *C. difficile* strains often circulate between people and farmed animals and that the zoonotic transmission is not restricted to any specific territory or the general populace. This clearly explains an inter-linked long-distance zoonotic transmission mechanism including potentially contaminated foodstuff and/or extensive dispersion of *C. difficile* in the environment. Indeed, research has demonstrated that RT 014 and other clinically significant and quite often antibiotic-resistant *C. difficile* lineages are found in retail food, waste, outdoor parks, domestic environments, and domesticated animals (Knight and Riley 2019). Now there raised a question that how this transfer of *C. difficile* between humans and food animals is possible with limited geographical clustering. Humans and animals both are the primary hosts for *C. difficile*. Since its spores can withstand the required cooking temperature for meat (71oC) for more than 2 hours, *C. difficile* from farmed animals may contaminate meat throughout the slaughtering procedure and survive until consumed by us (Rodriguez-Palacios et al. 2010). *C. difficile* spores may also spread via aerosols, as seen in hospitals and surrounding animal farm facilities. Based on regional agronomic systems and regulations, manure from livestock may be composted or used as organic fertilizer that is dumped directly into agriculture farms which might lead to contamination of the agricultural environment. It's doubtful that *C. difficile* spores will be eliminated from the manure, even after composting. Two-thirds of the composting material including mulches and soil mixture is still contaminated with *C. difficile* (Perumalsamy et al. 2019). Additionally, it is possible to compost contaminated food waste for later use in landscape and horticulture. Fig. 3 illustrates the important CDI reservoirs and potential transmission pathways.

When treated sewage is released, it may harm surrounding streams, lakes and rivers, and seafood (Troiano et al. 2015) because *C. difficile* can withstand the sewage treatment (Romano et al. 2012). There have been reports of direct zoonotic transmission of *C. difficile* between pigs and their farmer (Knetsch et al. 2011); nevertheless, for the public at large, indirect zoonotic dissemination via foodstuff and the environment seems to be more probable. Because *C. difficile* is so extensively spread in the population, residential settings and domestic pets are being colonized with *C. difficile*, opening up yet another pathway for transmitting CDI (Orden et al. 2018).

Conclusion

Treatment options for CDI have been limited to metronidazole and vancomycin since the late 1970s, when *C. difficile* was first identified as the causative agent. Consequences of CDI go far beyond gastrointestinal symptoms and are often overlooked. Recognizing the potential psychological and social effects of CDI and identifying payment assistance programmes, supportive services, and work medical leave options are all important parts of clinical treatment and management, but they are only part of a holistic approach that must take into account not only pharmaceutical intervention but the patient's experience both during and after CDI. Health resources and healthcare institutions should focus on avoiding CDI and CA-CDI to cut down on expenditures on medical treatments.

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29

Use of Natural by Products in Fish Nutrition

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INTRODUCTION

Fish are raised in captivity under strictly controlled conditions in order to meet the need of white meat for human consumption. In industrial fish farming, productivity is increased by making individual fish heavier (Schuchardt et al. 2008). Fish development with maximal weight in a short period of time is improved by using artificial feed in aquaculture (Bhosale et al. 2010). To increase the effectiveness of the feed conversion process, resulting in fish development, new compounds are being added to the fish feed. According to a study, herbs have positive benefits on fish health and growth. (Fernández-Navarro et al. 2006).

The formation of drug resistance bacteria and the generation of hazardous compounds unsafe to both human health and the environment are caused by the overuse of antimicrobials, hormones and other synthetic drugs in aquaculture to prevent infections, promote fish growth (Esiobu et al. 2002) and weakening the host's immunity (Panigrahi and Azad 2007). As a result, their use has drawn criticism across the world (Baruah et al. 2008). Herbs are used in place of antibiotics in the management of fish health because these are less expensive, environment friendly, and have less adverse effects. The World Health Organization promotes diets that include medicinal herbs or plants as a supplement to reduce the requirement for chemicals in fish meals (Dada 2015). In this situation, including herbs and herbal items in fish diets can help the cultured fish to consume more feed (Levic et al. 2008).

Animal nutrition uses bioactive substances found in a variety of plants to increase feed intake, enhance digestive enzyme output, and trigger immunological responses Additionally, there are many plants reported to have antiviral, antibacterial, and antioxidant properties (Citarasu 2010). Aquaculture practices include a diversity of herbs and herbal products to produce healthy fish by treating illnesses, promoting growth, lowering stress levels, enhancing immunity, stimulating appetite and preventing infections (Citarasu et al. 2001; Shalaby 2004).

Functions of Natural Products

Miguel et al. (1997) performed research on the dietary quality of cowpea seed protein concentrate as an ingredient in functional tilapia fry diets. Cowpea seed protein concentrate has been determined to be a potential element for use in tilapia feeding as there has been no documentation of mortality linked to dietary plant protein. Fish have benefited more from duckweed supplementation than from water spinach or other polyculture fish supplements (Thy et al. 2008). The results showed that effects of different amounts of *Ipomea batatas* peels on development, feed consumption, and specific cichlid biochemical responses of *Oncorhynchus (O.) niloticus* could survive up to 15% of inclusion of sweet potato peel. The research's findings are significant because it showed that sweet potato peel can be used in fish food to reduce the expenses of processing farmed fish. Similar effects of Tilapia fingerlings were exhibited by Adewolu (2008) and Faramarzi et al. (2012).

Mohddin et al. (2012) evaluated the impact of adding mushrooms as a prebiotic to a diet high in super worms on the growth of red tilapia fingerlings. Specific growth rate, Feed conversion ratio, and Protein efficacy ratio levels rose, and survival reached up to 93.33%. The 10% supplementation of mushroom stalk meal (MSM) as a tilapia prebiotic may be utilized in the insect-based diet of *Zophobas morio*. The Nile tilapia (*O. niloticus*) grows faster when the melon shell is included in its diet as a source of energy (Orire and Ricketts 2013). *O. niloticus* can be utilize up to 75% of mixing melon shell meal in tilapia diets successfully. The mean feed intake, survival rate, protein intake, protein efficiency ratio, gross feed conversion efficiency, feed efficiency and percentage weight gain all rose as the amount of dietary cowpea (*Vigna unguiculata*) husk meal increased. When cowpea hull meal is used in place of maize meal, growth efficiency improves by 50 to 100 times (Thy et al. 2008).

Dorojan et al. (2014) investigated the effects of specific phytobiotics (thyme, sea buckthorn) on the growth output of stellate sturgeons (*Acipenser stellatus*) in a system of economic recirculating aquaculture. Thyme (*Thymus vulgaris*) and sea buckthorn (Phytobiotics) were inserted in feed using gelatin at a concentration of 2% per kilogram of feed. The crude protein utilized was 48%. It was concluded that two types of essential plants (thyme and sea camel), when given at a concentration of 2% / kg of fodder, had an impact on astrocytes' ability to proliferate. The growth performance of tilapia (*O. niloticus*) fingerlings was studied by Gaber et al. (2014), in which diet were supplemented by Digestarom and it was concluded that the diet with 30% wet date and 0.03 percent Digestarom showed the highest net gain and looked to be preferably level.

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The effects of hunger stimulation on the functioning of digestive enzymes may be another potential mechanism of the action of phytogenic bioactive chemicals on the growth output of farm animals. According to Abdulrahman and Ahmed (2015), feeding animals fructooligosaccharides boosted their daily body weight gain by boosting their amylase and protease activity. Additionally, the pancreas and intestinal mucosa of these animals were used in a study that revealed feeding them a diet rich in lactic acid and a commercial blend of essential oils significantly increased the activity of digestive enzymes, which in turn significantly increased the production of pancreatic and intestinal mucosa enzymes. (Metwally 2009). It has been proven that using hot spices like cinnamon and pepper will cause salivation. The availability of nutrients from feedstuffs and digestibility may both benefit from the increase in enzyme synthesis (Yuan et al. 2008).

Higher vertebrates and fish have two critical components in their immune systems. The endogenous, regular, or non-specific defensive mechanism is the initial component. It is made up of a series of cellular and humoral elements. The second element is the acquired, specific, or adaptive immune system, which is distinguished by cellular immune response mediated by T-lymphocytes and humoral immunological response via antibody formation. Immunostimulant prophylaxis is the most successful techniques to improve the immune system for disease prevention in aquaculture. Some elements (such as the fundamental cells and molecules of adaptive immunity: B lymphocytes (B cells), T lymphocytes (T cells), immunoglobulins (Igs), and major histocompatibility complex (MHC) of fish's innate immunity have been found to be improved by immunostimulants (Esiobu et al. 2002).

Like other antimicrobial medicines, certain plant bioactive compounds work by altering the cell membrane of microorganisms. Minimum inhibitory concentrations (MIC50) and minimum bactericidal concentrations (MBC50) are correlated with the concentration of the active ingredient and the quality of the plant extract (Kamel 2001). Additionally, a significant increase in the hydrophobicity of microbial species can change the surface properties of microbial cells and therefore alter the virulence qualities of microorganisms in the presence of specific plant extracts (Miguel et al. 1997). This could be a key antibacterial mechanism in certain plant extracts. This description perhaps has impacts on the intestines, as certain pathogenic microflora depends on the adherence of bacteria to intestinal mucosal cells, which is strongly impacted by the hydrophobic surface features of microbial cells (Denev 2008).

Numerous essential oil blends that primarily consist of naturally occurring polyphenolic chemicals or flavonoids have shown promising results as antibacterial and antioxidant agents. A healthy gut flora can be established by supplementing broiler diets with essential oil blends, which will encourage optimal digestion and increase bird productivity (Denev 2008). Findings of Citarasu et al. (2006) showed that Black tiger shrimp's diet contains a variety of methanolic plant extracts. Shrimp fed a 25-ppm extract of turmeric fared which was substantially better against *V. harveyi*. According to another study, the ethanol extract of *Psidium guajava* was shown to have the highest antibacterial activity when it was tested against *Aeromonas hydrophila* infection in tilapia (*O. niloticus*) (Levic et al. 2008).

According to another experimental study, effective combination of plant extracts with antibacterial and antiparasitic characteristics is used to treat pangasius catfish and led to better disease resistance to two significant bacterial pathogens (*Edwardsiella ictaluri* and *Aeromonas hydrophila*), higher growth, and improved feed conversion.

From the experimental findings of Abdulrahman et al. (2019) it can be concluded that using 25-50% of the protein from commercial dry yeast *S. cerevisiae* in the diets of common carp led to a significant improvement in the chemical composition of common carp (*C. carpio* L.) meat, as well as an improvement in the sensory evaluation of fish meat.

By using a plant extract derived from the leaves of the olive tree (*Olea europaea*) and its primary component (oleuropein), salmonid rhabdovirus has successfully be controlled and viral haemorrhagic septicaemia rhabdovirus (VHSV) can be used as an antiviral agent (Dorojan et al. 2014). White Spot Syndrome Virus infection had an effect on the survival and viral load of black tiger shrimp fed on a combination of methanolic plant extracts (*Penaeus monodon*). The herbal medicinal products applied in *P. monodon* larviculture contain nutrients that enhance growth promotion, the appetizer to increase consumption and anti-stress characteristics and, therefore immensely used in the culture of shrimps. This practice reduces the side effects caused by applying synthetic chemical compounds. Hence the alternative herbal compounds prove to be very effective in the shrimp larviculture (Citarasu et al. 2002).

The outcomes of adding *Chlorella* algae to fish feed in the study of Abdulrahman et al. (2019) had an impact on feed consumption. The T3 and T4 food conversion ratios were noticeably lower than other therapies. No discernible variations across the treatments were seen in the food efficiency ratio. T3 and T4 had significantly higher protein efficiency ratios than other therapies. In both T2 and T3, fish weight without viscera was significantly higher. In the chemical studies (proximate analyses) of the common carp meat, T4 had a much greater protein to ash ratio, although T4 and T3 had a significantly larger lipid to moisture ratio. In comparison to the control, adding *Chlorella* to fish diets at all levels had a substantial impact on the color, juiciness, and overall acceptability of the meat.

When added to the diet of tilapia, the reddish hue seen in red sandalwood (*Pterocarpus santalinus*) is seen to boost the acceptance of the meal. Both the body and the flesh of the fish were painted pink. Fish fed colored feed displayed higher feed intake and growth rate (Gaber et al. 2014). Joseph et al. (2011) examined the effects of four herbal supplements (*H. rosasinensis*, *Rosa indica*, *Ixora coccinea* and *Crossandra* *infundibuliformiss*) on the development and body coloring of an ornamental fish with a red sword tail, *Xiphophorus hellerei* (Heckel), They came to the conclusion that *I. coccinea* had the highest amount of color pigments found in adult fish, at a level of 1%.

Jha et al. (2012) investigated that, natural pigments influenced cichlid color, and the groups showed no differences in feed conversion or growth rates. It was concluded as a result that these pigment sources affect the color of cichlid fish. With the addition of dietary supplements and an increase in marigold flower meal and beetroot meal, body carotenoid levels grow at linear rate.

Improving meat quality and aquaculture productivity depends on several variables, including density, the quality of the water and feed, as well as additional environmental variables such as the water's salinity and level of dissolved oxygen (Bhosale et al. 2010).

Environmental variables including rain, high temperatures, floods, landslides, predatory fish entering earthen ponds or reservoirs, blooming of algal, etc. are also the cause of a decreased aquaculture product. These elements lead to stress and insufficient usage of regular feed, which lowers the feed's conversion ratio. Drugs like antibiotics and synthetic medications that are used to treat illnesses can injure the liver and have an impact on the development of cultured fish, which lowers output. By addressing these issues, it will be possible to enhance commercially viable production. However, to maintain fish health overall and address environmental issues without compromising growth survival, enough herbal feed supplementation is necessary (Dada 2015).

Due to rising demand and a parallel decrease in wild fisheries, aquaculture is growing faster than all other animal food-producing sectors. With advanced cultivation/rearing techniques, numerous species of plants and animals continue to increase every year. Despite the various policy initiatives supported by numerous research studies aimed at boosting fisheries production in most countries, the deficiency in the production target is a key feature of the sector as the consumption of fisheries and products continues to increase. Production of aquaculture can be based on a short production cycle to fill the production gap. The feed conversion rate will be improved in this process, which in turn would encourage weight gain and boost the production potential of animal meat and have a bactericidal impact on wastewater aquaculture. Natural substances are promising to adjust the demand curve to fulfill existing demand in fisheries. Natural products may stimulate the growth of antimicrobial agents, increase the assimilation of proteins and the conversion rate of feed, so that animals gain weight more rapidly without substantial cumulative toxicity. Several herbs are used to lower stress, boost defenses, and control bacterial development, such as *Thymus vulgarism, Coriandrum sativum, Apium graveolens, Hygrophila spinosa*, *Withania somnifera*, *Zingiber officinalis*, *Solanum trilobatum*, *Andrographis paniculata*, *Psoralea corylifolia*, *Eclipta erecta*, *Ocimum sacnctum*, *Picrorhiza kurooa*, *Phyllanthus niruri* and *Tinospora cordifolia* (Citarasu et al. 2002). Similarly, it is known that in aquatic animals, fenugreek, black seed, licorice, anise, marjoram, caraway, basil, anise, fennel, and garlic boost development, food conversion, improve protein digestion, and sustain energy (Platel et al. 2002).

Significant improvements in fish growth, coloring, reproduction, and flesh quality can be achieved by adding *Spirulina platensis* to fish diets as a feed supplement or as a partial replacement for fishmeal (Abdulrahman et al. 2019). According to the latest research, common carp diets can substitute 20% of their fish meal with lentil seed for improved growth (Abdulrahman et al. 2021).

Allicin is an active garlic compound that causes increases in diet intake (Zeng et al. 1996). Research recorded that after 45 days of culture, the addition of 50 mg/kg synthesized allicin to the fish diet improve 2-3% of its weight gain (Lee and Gao 2012). As growth-promoting agents in *O. niloticus*, other culinary herbs including basil (*Ocimum basilium*), caraway (*Carum carvi*), and red clover (*Trifolium pratense*) showed promising outcomes (Ahmad and Abdel-Tawwab 2011). Another showed that green tea methanol extract (*Camellia sinensis*) improved the survival rate, growth, use of feed and protein content of black rockfish (*Sebastess chlegeli*). In *O. niloticus*, the supplemented diet of garlic increased weight gain and basic growth rate. High growth rates of 2.5 percent garlic in the feeding diet were observed in the same species. The diet supplemented with garlic increased weight and specific growth rate in tilapia. The 3.2% garlic powder diet showed the strongest performance in *O. niloticus* (Metwally 2009). Table 1 summarizes different prebiotics' effects on various carp species.

Similarly, diets that included garlic supplements showed significant advancements in weight gain, food conversion, and protein production. Due to increased protein synthesis, *Labeo rohita* fed with herbal diet supplements improved feed consumption, leading to better growth (Baruah et al. 2008). To improve fish health and wellbeing, earth apples were employed as a source of prebiotics which showed an improvement in weight gain and various growth parameters (Abdulrahman 2022).

Sesbania grandiflora, *Moringa oleifera*, *Coleus aromaticus*, *Ocimum basilium*, and *Solanum verbascifolium* leaves used to enhance growth in *O. mossambicus*. In a study, *O. Mossambicus* fed with diets containing *Moringa oliefera* demonstrated a maximum increase in weight gain. An increase in fish length was observed in fish fed with a diet supplemented with *Ocimum basilicum*. Additionally, in *O. aureus*, red clover (*Trifolium pretense*) mixed with diet promoted the growth rate (Levic et al. 2008). Juvenile pike perch (*Sander lucioperca*) supplied with control diets grew slower than those fed with diets supplemented with therapeutic herbs. Red sea bream, *Poecilia reticulata*, *Cryptoheros nigrofasciatus*, and common carp *C. carpio* growth was enhanced when medicinal plants were added to Pagru's main diet. Ginseng herb consumption accelerated the growth of *O. niloticus* fingerlings (Harada 1990).

Table 1: Effect of different prebiotics on carp species

Fish species	Prebiotic	Results	References
Common carp	Chitosan chitin	Increase in weight gain, lysozymes, Amylase and Lipase enhancement and increased defense against diseases	(Gopalakannan and Arul 2006)
Crucian carp	XOS	Increase in weight gain, and enhancement of Protease and Amylase activity	(Xu et al. 2009)
Common carp	MOS	Increase in condition factor, weight gain, and Protein efficiency ratio	(Atar and Ates 2009)
Crucian carp	MOS	Increased of immunoglobulin, lysozyme activity and acidophosphatase enzyme	Akrami et al. (2012)
Common carp	MOS+BG Immunogen	Increase in feed and Protein efficiency ratio, feed conversion ratio, Hemoglobin and plasma protein	(Ebrahimi et al. 2012)
Mirror carp	β- glucan	Increased heterotrophic bacteria, Increased growth performance, hemoglobin, hematocrit, and proximate analysis	(Kühlwein et al. 2014)
Common carp	FOS	Increased survival rate, growth parameters, white blood cells and diseases resistance	(Hoseinifar et al. 2014)
Crucian carp	Chitosan	Increased phagocytosis, Decreased cholesterol and triglyceride	(Chen et al. 2014)
Common carp	FOS	Increased survival rate, lactobacilli count, amylase and lipase activity, no effect on growth performance	(Hoseinifar et al. 2016)
Common carp	β- glucan	Increased microbial load	(Jung-Schroers et al. 2016)
Common carp	FOS	Increased white blood cells and platelets counts	(Abdulrahman and Ahmed 2015)

Table 2: Natural resources of natural prebiotics

Type of Prebiotic	Source	Reference
Maltooligosaccharides	Starch	(Kaneko et al. 1994)
Isomaltooligosaccharides	Starch	(Kaneko et al. 1995)
Raffinose oligosaccharides	Legumes, Lentil, Peas and Beans	(Johansen et al. 1996)
Xylooligosaccharides	Bamboo Shoots, Fruits, Vegetables, Milk, Honey and Wheat Bran	(Vazquez et al. 2000)
Galactooligosaccharides	Human Milk, Cow's Milk, and Glucan Dissolved in Water	(Alander et al. 2001)
Lactosucrose	Lactose	(Kawase et al. 2001)
Lactulose	Milk Lactose	(Villamiel et al. 2002)
Cyclodextrins	Glucan Dissolved in Water	(Singh et al. 2002)
Isomaltulose	Honey and Sugar Cane Juice	(Lina et al. 2002)
Palatinose	Sucrose	(Lina et al. 2002)
Fructooligosaccharides	Sugar Beet, Garlic, Dandelion, Barley, Onion, Honey, Banana, Sugar Cane Juice	(Sangeetha et al. 2005)
Soybean oligosaccharide	Soybean	(Mussatto and Mancilha (2007)
Arabinoxylooligosaccharides	Wheat Bran	(Grootaert et al. 2007)
Enzyme-resistant dextrin	Potato Starch	(Barczynska et al. 2012)

To improve growth effectiveness and meat quality, antibiotics may be given with an adjusted dose of garlic. Garlic was advised to be added to fish feed by Metwally (2009) to encourage growth and improve survival rates. Four distinct plants, including *Eichinacea purpurea*, *Allium sativum*, *Nigella sativa*, and *Origanum marjorana*, were utilized by John et al. (2007) as feed supplements to promote the growth and survival of *O. niloticus*.

Phytobiotics are defined as plant-based supplements given to animal feed to improve performance (Faramarzi et al. 2012). Phytobiotics have a broad range of properties, such as: growth promoters, antioxidant, anticarcinogenic, antimicrobial, analgesic, antiparasitic, insecticidal, anticoccidial, bile secretion stimulants and digestive enzyme function enhancement. In aquaculture, prophylactic administration of immunostimulants is among the most efficient methods of improving the defense system and control of diseases (Denev 2008).

According to a study, the treatment had a variety of impacts on the blood parameters that were examined, with 2.5 g/kg of fructooligosaccharide and dry yeast having a substantial impact on hemoglobin, white blood cells, and red blood cells (Abdulrahman and Ahmed 2015).

According to recent developments in immuno-nutrition studies, the immune condition of fish is related to particular nutrients. This brought the immune system protection and fish growth to the attention of fish nutritionists. Yuan et al. (2008) investigated the effects of common carp meals including a combination of *Astragalus Membranaceus* (root and stem), *Polygonum multiflorum*, *Isatis tinctoria*, and *Glycyrrhiza glabra* (0.5 and 1%). The levels of total protein, albumin, globulin, and nitric oxide activity increased significantly with both amounts, but there were no statistically significant differences in the activities of superoxide dismutase, lysozyme, or triglyceride levels. Fish immunological function has been reported to be improved by the root extracts of the Chinese herb Astragalus, which contain organic acids, alkaloids, polysaccharides, glucosides, and volatile oils as its main active ingredients. The oriental medicinal herb *G. glabra* (liquorice) comprises flavonoids and pentacyclic triterpene saponin, including liquiritin, liquiritigenin, isoliquiritigenin, liquiritin apioside, glycyrrhizin and glycyrrhizic acid as major constituents and is reported to have antioxidant effects (Yin et al. 2011).

The usefulness of adding herbs to fish feed to control abnormalities and promote healthy fish is the subject of numerous research. Fish feed can benefit from the use of natural plant products for improved development, health, and productivity. Aquacultural activities that use herbal products can promote development, operate as immune system boosters, stimulate hunger, raise feed consumption, induce maturation, and have antibacterial and anti-stress properties. The application of plants may pave the way for better coordination of fisheries with horticulture, providing a better connection between aquaculture and agriculture and achieving the objective of increased aquaculture production in a sustainable, cost-efficient, and eco-friendly method (Abdulrahman 2022), as shown in Table 2.

Green herbs and herbal items have been added to the feed to help fish raised in a farming system to resist infections, promote growth, reduce stress, and boost their immune systems. Diet supplementation of fish with herbal products is more affordable and environmentally safe, and neither the fish nor the consumers will experience any side effects. As a result, using herbs as medications to treat ailments in aquaculture is becoming more and more common. Compared to the numerous medications and vaccinations used in disease prevention, these have a greater impact on safety (Abdel-Tawwab et al. 2022).

Conclusion

It is evident that plant by products can affect physiological functions and modulate the intestinal microbiota of different animal species, showing potential to be used as growth- and health-promoting agents in fish production. Natural products can be used as growth promoter, immune-stimulants, appetite stimulators, antibacterial, antiviral, antifungal, and anti-stress agents. Further studies are needed to gain more insights to understand the mechanisms of action of various by products on both pathogenic micro-organisms and the more abundant normal microbiota of the gut and evaluate their impact on the environment and the host.

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Camel Production Profile and Role in Food Security

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INTRODUCTION

Emerging goals and a growing focus on the camel have modified the belief of "supply of the barren region" to "a food safety animal" in lots of semi-arid as well as arid zones of the world. Pakistan isn't an exemption to this – wherein the camel goes to take part in a pinnacle characteristic that may fulfill all requirements of milk and meat of the rural population. The saying of the Almighty in the Quran "Do they not have a look at the camel, in what way they may be made"; nicely extolled the virtues of the camel (Al-Ghashiyah, 88). It is thought that after the barren area residents proceeded to God and complained about the difficult climatical situations of the desert, God heard their pleas and sent them the female camel, they drank its milk, and their characteristics grow well (Khan et al. 2003). The exploding population will increase the priority of food safety around the globe wherein Pakistan isn't an exception as well, as the country is 5th largest regarding population and is challenged with food safety (Faraz et al. 2019a). In the investigation of the latest property, a future need is the camel as its far vital count in the proper food chain system (Faraz et al. 2019b).

Food protection has originated to be excessive trouble worldwide because of the increase in the population of humans. To contest this condition, the investigation of novel worldwide properties is a dire need of the current situation. The camel looks quite a central count to the proper food chain because it gives meat, milk, and important way of financial significance (Faraz 2020a). It is a key supply of subsistence and earnings for pastoral human beings (Faraz et al. 2019c). Commonly, there are two types of camels i.e., "*Camelus dromedarius*" called one-humped camel or may be called Arabian camel, and "*Camelus bactrianus*" called two-humped or may be called Bactrian camels (Faraz and Waheed 2019). There is a 35 million worldwide camel population and at the same time Pakistan ranks eighth in the globe with 1.1 million camels after Somalia, Sudan, Kenya, Niger, Chad, Mauritania, and Ethiopia (FAOSTAT 2018; GOP 2021-2022).

Camels Distribution

One-humped camels are 95% of the total camel population. There are near about 50% of the dromedary camel population belongs to Somalia and Sudan and 70% of the Asian dromedary camels belong to Pakistan and India. Baluchistan consists of about 40% of the camel population in Pakistan; about 30% in Sindh, almost 023% in Punjab, and about 07% in KPK province (ACO 2006). Dromedary camels have dominated the camel population in Pakistan as some herds of Bactrian camels are inhabitant at Suleiman mountainous region (Raziq et al. 2010).

Current Status

There are 4 environmental regions of camel production in Pakistan. Thal and Cholistan (sandy barren vicinity) in Punjab, Costal areas of Sindh province (Badin, Karachi, and Thatta districts), Thar in Sindh and Hilly zones (Dera Ismail Khan, Dera Ghazi Khan, of Punjab and many areas of KPK, and Balochistan). There is camel production in different watered districts of Sindh and Punjab provinces as well (Faraz et al. 2013). Camelids can be multi-purpose organisms whose female animals are used generally for milk production, the males are raised for meat purpose, draught, or transportation cause, and all over again each animal either male or female provide meat as a tertiary product (Faraz 2019a).

Camel has been considered a very important animal in many countries which is used as a food animal now-a-days because of the growing recognition of meal nutrients and dietetics. Its functionality to use roughs of rangeland in borderline zones and to live on and bring properly underneath warmness and cruel surroundings have been diagnosed considerably over the years. Globally, camel attains a completely specific socioeconomic and ecological reputation (Faraz et al. 2019d).

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Its important feature is to provide benefit to the poor people though multiple ways and can survive severe climatic conditions since historic times (Faraz 2019 a, b, c). The camel has several unique qualities, including the ability to cruise, be milked, carry loads of goods, eaten, be secured, plowing, traded for goods, and employed aimed at unusual agronomic tasks, as well as being displayed in zoos (Iqbal 1999).

Camel Peculiarities

There is a significant role of camel in the subsistence pastoral economic system in a number of ecozones ranging from India and the Desert Gobi in first-world Somalia to Asia and up to Ethiopia in the base of African continent (Kohler-Rollefson 2005). The ability of camels to survive in difficult, dry environments and to withstand a variety of pressures, such as heat, a lack of water or water with excessive salinity, and a lack of food, has made it a special candidate pillar of marginal ecosystems (Faraz 2020b). It can digest dry roughages and depends further to consume all cheap and rough nutrients especially crude fiber greater than one in each of the ruminant types. The extreme digestibility of fiber might be recognized as the lengthier maintenance period of enormous debris in its front belly as well as a sole measure of the fore-stomach of the camels. The camel (dromedary) is the maximum crucial element of domestic animals within temperate, semi-arid, and dry areas because it has the ability to deliver outstanding food like milk and meat in severe climates at decreased prices. It has a great tolerance to the higher temperature, solar radiation, and water shortage. It is capable to thrive nicely on sandy deserts with terrible flora and especially consumes the feed material that is unutilized with the useful reserve of many precise domestic animals (Faraz 2022).

In Pakistan, Camels are very well adapted to their close surroundings and may preserve their life cycle in warmth and severe deserts. The camel (dromedary) is an excellent supply of milk and meat, particularly for the regions wherever the production system is advanced and it can bear harsh ecological conditions of the weather. This reason is its sole physiological predisposition which allows bearing higher temperatures, water scarcity, solar radiations, dreadful flowers, and challenging geography. These animals have no antagonism with local species concerning feed and are well known for extensive performance. They may be browsers as their loosened lips with strong papillae and buds in addition to prolonged legs and neck permit the animals to browse proficiently. As concerning their detailed characteristics, the animals shall browse that feedstuff cloth and this can be an outside technique of many farm animal species (Faraz 2020 a, b).

A substantial portion of camel’s milk is there in cardinal provisions of rural community which pays for about 30% of the yearly energetic diet. On an average, the everyday milk production of she-camel is normally 3-10 liters in subsistence systems and a longer lactation length of 12-18 months (Farah and Fisher 2004; Ahmad et al. 2010). At standards, on average camel milk retains 3.7% protein, 4.9% fat, 5.1% lactose, 14.4 % total solids, and 0.70 % ash (Khan et al. 2005). Definitely, the milk of camel is enjoyed now, due to its richness with nutrients and medicinal worth. Now society starts using it as therapy for certain conditions like diabetes, liver dysfunction, tuberculosis, long bone pain, asthma, spleen ailments, piles, arthritis, food allergies, and sometimes as an aphrodisiacal. Furthermore, the milk of camel has high level of Phosphorus and vitamin C contents (Konuspayeva et al. 2011), therefore it has an advantage over other domestic species for milk comparison (Saini et al. 2007; Mohamed et al. 2005).

Camel Accredits

The camels haven’t any opposition with other home species about feed and continuous production. The grazing and browsing comportment of camel consists of actions pattern which is linked to the searching, choosing, ingestion, and absorption of feed (Faraz et al. 2018a). According to some experts, the camel has the ability to graze on high, more beginning, and moist grasses but usually, it's miles a browser and their feed includes timber, shrubs, and wood (Faraz et al. 2018b). Camels are browsers by nature as it is tall in addition to prolonged neck and legs allow them to browse effectively. Because of those specific qualities they will be proficient in browsing that feeds fabric that is away from the method of diverse livestock species. They shall browse on sensitive flora, and salty and prickly timber because of the specific shape of their mouth. It has robust prehensile lips, papillae, and a thin muzzle which allows it to browse appropriately on thorny foliage (McDowell 1986).

Camels like browsing as compared to grazing and it is declared as a browser with the beneficial resource of nature (Yagil 1990). Camels spend more time in rumination that is why they select surfing overgrazing (El-Badawi 1996). In the absence of marvelous forages, camels can make usage of terrible bizarre forages with greater effectiveness to keep fibers in their fore-stomach for a longer time of about 70 hours. Even described by Schwartz et al. (1992) in the assessment to one-of-a-kind ruminants, the feed of camel is consisting of forage with low protein as the camel has an ability and overall performance of reusage of the urea for synthesis of microbial/stomach protein far better. As one of its attributes, the camel is in consideration because of the authenticity of the animal with unfathomed volume to meet the purpose of dietary and medical requirements of people resident in the marginal areas (Faye and Esenov, 2005). But, in regardless of a selection of these traits, it has been continued to be an unobserved animal.

In arid regions, the camel signifies the maximum vital meat supply line (Knoess 1977; Farah et al. 1992). Camel is usually raised up at conservative control structures as pastoralists are moving continuously for handsome water and meals over enormous zones for the camel (Abbas and Omer 2005; Omer et al. 2008; Ali and Majid 2013). Camel inherited native beneficial aids for easy management and conserved nicely. Camel achieves an indispensable purpose in rural biology. Good research spotlight its sole propensities, particularly under pressure situations. In order to satisfy, the emerging needs of the explosion population, the planned knowledge is needed to lessen the need for exterior provender.

Performance Outline

Camels play an indispensable position in the community survival and economic device of human beings insemi-arid and arid zones in numerous areas. No matter the situation great input to the income of the rural community, camel is the maximum abandoned species and just rare effort had been required to represent its performance ability and interrelated limits under natural situations. At the same time as in predictable control tools the camel well-organized inclinations are short, and the old-style farming of camel has no forthcoming (Bakheit et al. 2012).

The farming of camel structure is in flux position because pastoralists are shifting from their outdated complex to intensive and semi-large arrangements. The abruptly converting situation desires popular assessment and a pressing wants to embrace multi-disciplinary kinds of research (Khan et al. 2003). Basically, the studied artwork on the industrial capacities of the camel is accomplished below conventional manage structures without notice of producing structures in Pakistan (Iqbal et al. 2001). Research artwork finished so far is totally on ever-changing herds and a lot of paintings have been based totally on survey studies below traditional control structures. The studies about the role of camel in the massive famine are positive and the basic facts on Pakistani camels and their developing functionality exploitation are critical to carry together with a rustic records base for destiny studies and to search for export potentials. Presently, Faraz along with his coworkers has completed large research concerning production capability of camels, in particular, in the manipulation systems in Pakistan, at the manner to pave a way for further investigations in this hassle and concluded that the camel appears to have a very vital chair for the food safety in the country and it is the precept pillar of the monetary device in marginal compass (Faraz 2019a).

Camel is a source of milk, wool, meat, hair, and conveyance. Camel assists hundreds and thousands of human beings in semi-arid, mountainous, arid, and particularly barren regions of our country. It has specific functional tendencies. The camel has one specific full of fat-rich parts of the body called humps on its upper part which have the ability to store calories for the camel inside different directions of the dearth of the water source. Camel's hump does no longer consist of water; in fact, its miles save of fat on which the animal can live at the equal time as food is scarce (Faraz et al. 2013).

Many types of camel breeds are determined in Pakistan. Mountainous camels regionally known as Pahari, are inhabitants of Northern Punjab as well as in Balochistan. The riverine type of camels may be placed in the barren area and irrigated grasslands of Sindh and Punjab provinces. Twenty different camel breeds are determined in Pakistan that includes 7 breeds of Balochistan (Lassi, Brahvi, Pishin, Makrani, kharani, Rodbari, and Kachhi), 4 of province Sindh (Sindhi or Larri, Kharai, Dhatti, and Sakrai), 4 of the KPK (Gaddi, Ghulmani, Khader and Maya) and 5 of Punjab (Booja or Bagri, Cambelpuri or Mountainous, Marecha or Mahra, Kalachitta, and Thalochi or Brella) (Faraz et al. 2013).

Three important production systems of camel are present in Pakistan; transhumant, sedentary and nomadic. Those performance systems in truth represent the socioeconomic significance of camel as those are associated with climatically situations, flora phonology, topography, and availability of water. In nomadic systems, raising camels is frequently compared to human social lifestyles. Nomads travel from their homeland to different areas and the principal motive for their roving is the absence of fodder for grazing and water scarcity troubles. Camels, about 26% of the herder areas in Pakistan, have a test of this type of production tool. These tools have been characterized through 3 primary capabilities: 1) Herds of camel are numerous with the useful resource of goat, sheep, and donkeys, 2) Seasonal and catastrophe movements are obvious, with survival being the most important factor, 3) Same traditional activities adopted by the nomads who lend and share the camel herds (Faraz et al. 2019 b).

The transhumant system may be changed cultivation processes inside the rainy area at a few unspecified times. The migration is also based totally upon the important purpose of accessibility of feed and water. In Pakistan, 23% of farmers are concerned about transhumant technology. Nearly 50% of the population is employed in the sedentary activity of camel rearing, which accounts for a significant portion of family income. Women play a fantastic role in this tool. Now they may not be best involved within the rearing of camel however involved in converting the byproducts into beneficial merchandise and market them (Faraz et al. 2019 a).

Camel Benefits

Pakistani camels are widely known and renowned for their ability to produce milk. Lactation period of 270-540 days and well-known milk production tiers ranging from 1300-4200 liters, Marecha is likely the world's best milk yielder with a mean milk yield of 4179 liters per month. In parts of terrible fodder/feed and in barren region situations, reports showed that the regular production is about 4 liters/day and most heavy camel breeds can also produce 35 liters constant with the day. Unlike other domestic species, the camel can keep an average yield for a long term (at a minimum for 365 days) by providing sufficient water and feed requirements (Faraz 2020 a).

The milk of camel is superior to the milk of several domestic species because it is richer in minerals, fat, and vitamins, particularly vitamin C, and protein. It is far wealthier in mineral phosphorus and contains numerous additives (Konuspayeva et al. 2009; Konuspayeva et al. 2011). The self-life of camel milk is better because it has better contents of protein that accomplish a repressive movement in opposition to pathogenic bacteria. So, milk is directly sold in vital disinfected situations even at high temperatures (Faraz et al. 2018 a).

Up-to-date farming techniques allow for commercial dairy husbandry of camels, particularly in areas of the Thal and Thar deserts and Cholistan rangelands. The milk from camels is consumed raw or made into yogurt. Camel milk has particular special preferences, and Pakistani camels in particular possess all of the outstanding dairy abilities that make them fantastic dairy animals (Faraz et al. 2019a). With the day-to-day farming practices of camel, it can deliver for forthcoming food production systems, particularly in semi-arid, arid, mountainous, and barren regions (Faraz et al. 2017). Normally for Pakistani camels, the milk production is 2920 kg with an average daily yield of 8 kg which is far better than countries like India, Somalia, Tunisia, Algeria, and Ethiopia where it is about 4-6 kg on an average (Faraz et al. 2013).

Like many unique breeds of cattle, camel calves have wonderful growth (Faraz et al. 2017; Faraz et al. 2018b). In Pakistan, the ancient use of camels is typically for slaughtering purpose while only a few castrated camels are employed for fattening purposes. During Eid-ul-Ezha, Muslims joyfully slaughter camels because they are a valuable resource and they can sell them for a very high price. Opportunities exist for export to Egypt, Saudi Arabia, Libya, the Gulf, and particular Arab states. In Pakistan, specific slaughterhouses slaughter between 100 and 150 camels every day. The meat of camel is eating up as sparkling, in minced shape, and furthermore in sausages and barbecues. The taste is much like meat/beef from cattle. The quantity of protein, minerals, and ash is equal to that of cattle meat; however, the meat of camel contains very lesser amount of lipids and extra water contents. The dressing percentage may go up to 60% with a lot of healthy meat (Khan et al. 2003).

An adult camel typically produces 1-3 kg of hair per year and these hairs are used to make ropes, carpets, luggage, mats, and blankets. Additionally, it produces exceptional wool, particularly the first fibers sheared from newborn calves, is used to make blankets. Saddles and shoes are made from their hides at the same time. The usual fee for its coverage is PKR 3000-4000, but occasionally it can be as high as PKR 5000-7000 (Khan et al. 2003; Faraz et al. 2013).

Mantle Role as Food Resource

The camel has ended up nicely to be referred as a great animal in barren land conditions. Now the animal is playing a vital feature in several atmospheres. Camel gives valuable food and has a weird feature inside the food chain. It has the supply of meals to the people living in the harsh weather especially to the deep abandoned population of marginal areas wherein there's immoderate competition for feed among animals. It has the capability to carry out and thrive well within one's climates wherein even the survival of numerous cattle species is difficult as the production is involved. Those virtues have given the animal an eager significance that it could be the future need to bridge the triumphing food scarcity for the population. Pakistan is not an exception to the exchange, in which the notion "deliver of the barren place" has been shifted to "food protection animal" on behalf of camel. Camel is of top importance and has a fantastic supply of meals and socioeconomics in deserts and mountainous areas. Camels meet the keen requirements of milk and meat of human beings and because of this it serves as a meal's safety agency within the region (Faraz et al. 2013).

Conclusion

In conclusion, the camel has an extreme significance and contributes a key part in the people's social livelihood as well as in the environment of arid region. It can satisfy the milk and meat needs of pastoralists, nomads, and rural inhabitants. Therefore, it is by far the best candidate for food protection in changing climates in the environment of the desert as well as in many Eco zones.

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Toxic Trace Elements in The Environment and Their Effects on Human Health

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INTRODUCTION

The human population is snowballing and developing into modern societies. The surrounding of humans' external factors including living and non-living objects or materials is called the environment. (Seinfeld 1989) The environment has been divided into physical (water, soil, air, housing, radiation, etc.) biological (Plants, animals, microbes), and social factors (norms, values, culture, income, habitat, occupation). Excess of elements in the environment is called pollution or contamination. Modernization is a significant cause of environmental contamination. Modern industrialization converted farms into urbanization and industrialization. (Kampa and Castanas 2008) The combing effects of industrialization and urbanization exacerbate environmental contamination, which causes deleterious effects on human and animal health. Environmental contamination is a worldwide issue since the beginning of the 20th century. But, developing countries face more problems due to less control of developmental plans and their implementations. (Kramer et al. 2021) Rapid population growth and inadequacy of resources boost the situation. The most dominant effect of pollution is water and air pollution. Human health is mainly dependent on the environment. (Xie and Zhu 2020).

History of Environmental Contamination

Before 1000 CE coal was used as fuel, and the conversion of coal into gases was probably the first human action that destroyed the surrounding. At the beginning of the 17th century, the era of industrialization and urbanization started which was the major source of congestion. (O'Neill et al. 2003) The proliferation of the population boosts the situation in the environment. By the end of the 20th century, pollution became a global problem. After the World War II, the Japanese faced health problems, because of post-war environmental effects, and radiation exposure These terrible effects lasted many decades but, they made plans to evacuate the situation and emphasis on environmental control strategies (Mayer 1999).

In 1962, environmental safety precautions started at international level for balancing the pollutants in the environment. In 1972 the UN conference on humans and their environment facilitates a wider survey to assess the environmental contamination (Bai and Ogbourne 2016). Table I shows various histological events regarding environmental pollution. Ecological maintenance means environmental health, which includes primary prevention of diseases and improvement in quality of life.(Martins 2010) Essential elements include water, air, and sanitation. More than 2 billion people in the developing world have no access to a safe environment. The attainment of healthy environment is more complicated in the current situation (Briggs 2003).

In 1955 first Federal Control Act had passed to control and restrict the pollution by authorized state and local agencies. In 1967 Air Quality Act passed to check, maintain and measure the pollution causing agents and their control (Sun 2020). In 1970 Environmental Protection Agency passed Clean Air Act to deal with new industrial cities near populated areas against plants to protect environmental contamination (Khan and Ghouri 2011).

Major Environmental Pollutions involves

1. Air pollution
2. Water Pollution
3. Land/Soil pollution
4. Noise pollution

Air Pollution

Presence or introduction of substances, which cause health hazards in the human, animal, and other living lives known as air pollution. There are different sources of these pollutants, most importantly atmosphere is sometimes acts as a self-pollutant.(Seinfeld 1989)

i. Primary air pollutants

Exhaust or chimney of the factory and suspension of dust particles from industrial areas are major sources of primary pollutants (Calvert and Englund 1984).

ii- Secondary air pollutants

Secondary air pollutants form when different primary particles present in the air chemically react with each other in the atmosphere, the most common example is ozone, which arises from different chemical toxins pollutant (Redekop 2010). Those pollutants which are present in gaseous form in the atmosphere. It may be a small molecule,

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Table 1: Different historical perspectives of environmental pollution (Briggs 2003)

1	The first action was taken by the population	1868	The start of noticeable pollution in the environment
2	Before World War I (the era of industrialization)	1869-1914	Chemical exposure in the environment through industrialization
3	First World War	1914-1920	Use of weapons created congestion in the populated cities
4	First to second world war (industrial revolution)	1920-1945	Exposure to radiation, nuclear weapons use.
5	Population rapid growth	1945-1970	Urbanization causes congestion in cities
6	Control and preventive measures started through international organization	1970- 2000	Laws to reduce pollution

having ability to cross filters and can be inhaled through respiratory system. These gases are major sources of upper respiratory tract infections such as Asthma, bronchitis, allergic rhinitis etc. It includes carbon mono-oxide and nitrogen dioxide etc.(Burke et al. 2005).

iii- Particulate air pollutants

A solid form of pollutants presents in the air but in very low quantity. These pollutants cover the wide range in the atmosphere. These smaller particles may be 1-3 micrometer in diameter (Peirce et al. 1998).

iv- Local-scale pollutants

Some pollutants or particles having very short lifetime and may convert into other substances. These are easily encountered but poor countries have no resources to encounter these particles. For example biomass for fuels, burning of coal produces gases and solid particles (Peirce et al. 1998).

v- Urban Scale Pollutants

Some pollutants significantly produced in urban communities which is highly produced by traffic. It includes nitrogen oxide, free lead, cadmium etc. These pollutants have longer lifetime and stay in air, which causes respiratory and ophthalmic disorders (Hirsh 2014).

vi- Global scale pollutants

Accumulation of pollutants leads toward global damages. Pollution is now a worldwide problem (Alloway and Ayres 1997).

vii- Metrological factors

The atmosphere extends into several layers and surround 30 km above the sea level. Man, directly interact with 8-10 km above sea level. Air movement, climate, temperature, dilution, and dispersal of atmosphere more significantly known as topography. The diffusion of pollutants into the air is highly dependent on these factors. Topography may be dominated by mountains, high buildings where it became calm and weak. Pollutant tend to concentrate because of topographical factors which causes breathing zone difficulties (Giesy et al. 2010).

Sources of Air Pollution

Air pollution considered into two major categories

i) Natural air pollutants which include wind-blown dust, volcanic gases and ash, natural radioactivity, pollens, gases from natural decomposition and Ozone-depleting layers (Hirsh 2014).

ii) Manmade air pollutants are industrial pollutants, automobiles, combusting processes, plans, aerosol cans, incinerators, petroleum refineries, steam generation and copper smelting. All these sources major contributor of air pollution (Vesilind et al. 2013).

Classifications of Air Pollutants

Air pollutants can be classified into two categories. One is the origin of pollutants and the second is the state of pollutants (either liquid, solid, or gaseous).

(i) Particulate Pollutants

The pollutants in a liquid or solid state are considered larger than a molecule or vapor. They dispersed in the form of aerosolized particles like smoke, fumes, dust, haze, and fog. The size of these particles varies from 1 micrometer to 100 micrometers. Fine particulates settled down too slowly, while heavy settled easily (Orlitzky et al. 2011).

The metallic emission produces trace elements such as mercury, lead, cadmium, zinc, selenium, manganese, and nickel. These heavy metal evaporators cause mutation in genes and produce carcinogenic effects.(Xu et al. 2018)

(ii) Gaseous Pollutants

The release of gaseous substances into the air directly interacts with the human respiratory system which produces deleterious effects. Most of the gaseous pollutants originated from the combustion and industrial process. Gaseous pollutants can be divided into organic and inorganic pollutants. (Burke et al. 2005)

Organic Pollutants

Oxygenated organic compounds are:

i) Alcohol
ii) Hyper oxides
iii) Aldehydes
iv) Ketones
v) Cyanogen chlorides
vi) Bromobenzyl cyanide
vii) Dimethyl sulfide
viii) Acetones

Inorganic Pollutants include

i) Paraffin (Methane, Ethane, Octane)
ii) Acetylene

iii) Olefins (Ethylene, butadiene)
Aromatics (Benzene group, Toluene, Benopyrene) (Dourish 2010)

Indoor Air Pollution

Indoor air pollution contains a variety of contaminants which is majorly categorized into physical, biological, and chemical pollutants. These contaminants arise from multiple resources. Indoor pollutants categorized into two groups. One is most commonly causing discomfort and acute illness and second that causes chronic illness or carcinogens (Dourish 2010). Micro-organisms causes acute allergic reactions. If it remains persistence it will convert into poor chronic health. Indoor pollutants classified into i) occupational activity ii) wood combustion iii) construction materials iv) furnishing v) garages. (Xu et al. 2018) Temperature and humidity effects the contaminant's saturation. Elevated temperature boosted the volatile organic contaminants in the air. Humidity aggravates particular matters and release of heavy matter in the air (Fayiga et al. 2018).

Combustion Process and its Effect on the Environment

Combustion process is the most common source of indoor air pollution. Kerosene, wood, coal, and oil generally produce a greater quantity of pollutants. These substances produce suspended particles in the air which are contributing to respiratory problems. Table 2 depicted the amount of emission per milligram per hour during the combustion process (Edwards 2013).

Air Pollutants Responsible for Air Pollution

More than 120 substances are responsible for air contamination and health hazards including carbon monoxide, nitrogen dioxide, sulphur dioxide as organic substances while inorganic substances include arsenic, lead, zinc, photo chemicals, chloride, fluoride, mercury, benzene, asbestos, ozone, chlorine fluorocarbons, and other radioactive compounds. The combination of smoke that reacts with suspended particles in the air form smog (Khan and Ghouri 2011).

Carbon monoxide: The most common and widely distributed air pollutant produced during the process of combusting of oil, carbon materials, industrial heating generators, automobiles, hospital incinerators and other oil-based machinery. Incomplete fossil fuel burning produced carbon monoxide. The concentration of carbon monoxide is greater in urban areas. The reason behind the high saturation of carbon monoxide in heavy vehicles, traffic high density, and emissions from small industries. Spikes occur during the evening hours in cities. It directly influences humans by producing carboxyhemoglobin. The high concentration of carbon monoxide attaches to hemoglobin in replacement of oxygen, which leads to multiple health disorders (Briggs 2003).

Lead: Second most common and most dangerous micro-pollutant is lead. Free lead produces from automobile fuel combustion. The degree of lead pollution varies from country to country. Lead pollution is more saturated in developing countries, while developed countries use lead filters and lead-free petrol (Downs 2016). Another most common cause of lead exposure is the smelting and mining of lead that creates pollution locally. Lead pollution in children causes behavioral changes or growth retardation. In adults, it causes lung disorders and skin irritations (Mayer 1999; Briggs 2003).

Hydrocarbons: Manmade activities and machinery producing excessive hydrocarbons. It releases in the environment leads to hydrocarbons pollution. Automobiles, coal and combustion, incineration, and local industries are liable for contamination of hydrocarbons. These hydrocarbons react through a photochemical process and produce smog (Berry 1977).

Sulphur dioxide: Sulphur contains in multiple forms including H_2S, H_2SO_4, and SO_2. Sulphur dioxide is a colorless gas that is excreted from industrial fuels, coal burning, motor vehicles, and domestic wood fires. Sulphur dioxide causes respiratory disorders, irritation in the eyes, asthma, chronic bronchitis, coughing, mucosal secretion, and lower respiratory infections. More than 10 minutes of direct exposure to SO_2 causes dyspnea and tachycardia. Sulphur dioxide combined with water and produce H_2SO_4 which is the major component of acid rain (Ren et al. 2011).

Carbon dioxide: It is not considered a pollutant, but anthropogenic activities produce more carbon dioxide through the combustion process. It does not take part in any chemical reaction because it is a natural constituent. High carbon dioxide raises the temperature of the earth (Moldan et al. 2012).

Cadmium: In the steel industry, cadmium and zinc produces during the process of incineration. As tobacco contains cadmium, smoking raises the level of cadmium in the environment as well as in human lungs causing lungs damages (Azam 2018).

Oxides of nitrogen: The burning of coal, petrol, and the process of electricity generation produce nitrous oxides, which are responsible for a reduction in lung functioning, and growth (Malhotra et al. 2013).

Table 2: Indoor combustion emission of pollutants (Edwards 2013)

Equipment	Carbon Monoxide	Nitric oxide	Volatile organics	Aromatics	Particulates	Sulpher dioxide	Formaldehyde
Oven	1898	55.5	-	-	0.13	0.9	22.9
Gas heater	2659	-	-	-	0.38		41
Good radiant heater	588	4.8	16.2^5	0.06^4	00.01	34.3	
Poor Radiant heater	138	148			0.28	45.5	41
Airtight stoves	11-160			0.0001^3	$2.5\text{-}8.7^2$		
Non-airtight stoves	252-618			0.08^3	16-73		

Particulate matters: It is the complex mixture of organic and inorganic substances, including particles larger than 2.5 mm but small and fine particles less than 2.5 mm. Particulate produce in the process of burning fossil fuels, and industrial incineration. These particles reduce the growth in plants and bronchioles destruction in humans. Chronic exposure to particulate matter causes cardiovascular diseases as well as respiratory diseases. It is majorly responsible for lung cancer (Bilen et al. 2008).

Measurement Methods of Air Pollution

Quantitative analysis is the effective and easiest method to assess the quality of air. Accurate sampling after study design sets a criterion for control. Sampling location is the most important step to evaluate the actual contaminants of the area. For example, in a cross-section following calculation is used to calculate an accurate sample size (Bask et al. 2018).

Measurement of Gases: A probe fitted with an integral filter is used to collect samples. This filter removed the particulate from the air as well as minimizes the loss of gaseous substances. Cooling methods are generally used for collection of samples. The knowledge of molecular weight and the actual velocity of gaseous substances is necessary to collect samples. There are following methods used to evaluate or analyze the gases amount in the air.

Major gaseous constituents are

i) Carbon monoxide
ii) Sulphur dioxide/ Sulphur trioxide
iii) Nitrogen dioxide
iv) Hydrocarbons

Photochemical oxidant (Long et al. 2021)

Chromatographic analysis: Sampling can be done through the collection in the glass or plastic bag. Following factors should be considered during the evaluation

1. Specificity (the instrument's response to other substances encountered in the sample).
2. Precision & Accuracy
3. Stability
4. Sensitivity
5. Sampling average time
6. Reliability and economic feasibility

For continuous monitoring, there should be further consideration

- Zero calibration and drifting
- Response, lag, rise, and fall time accurate changes measurements.
- Ambient temperature and humidity
- Maintenance requirements

Machine-readable format to check data output (Gómez-Puerta et al. 2021).

The standards of air quality assessment have been described in Table 3.

Measurement of Particulate Pollutants

There are different methods to measure the particulates in the air. The commonly used methodology includes i) sizing of particulate ii) mass measurements iii) opacity

Sizing of particulate: There is no single unit of measurement of a particulate size. Size can be measured on the basis of linear dimension, projected area, surface area, volume, and mass. Some measurements of particulates are mentioned in the Table 4. By using the physical sizing method, the particulate size is determined (Khan et al. 2019).

The Effect of Trace Elements on The Human Health

Well-being and human health are largely dependent on the surrounding environment. Multiple researchers working on the relationship between humans and the environment from the last two centuries (Khan and Ghouri 2011). Human health is majorly affected by numerous environmental factors, including what a person drinks, eats, lives and breath? The environment provides energy to humans in form of water, air, food, and exposure to sunlight. When there are micro-contaminants in the air, water, food, and sunlight it will produce an imbalance in the metabolism or normal mechanism of action in the human body. Significantly, pathogenic microbes are responsible for the disease process (Schweitzer and Noblet 2018). On the other hand, various human inventions and activities are also boosting the disease process. Occupational stressors in industrial cities disclose the hazardous material produced in drinking water contamination, noise pollution, high temperature, poisonous chemicals, pesticides, fertilizers, and pressure leading to pathological conditions (Bashir et al. 2020).

Anthropogenic and Environmental Challenges

Population explosion, progressive economic developments, natural resource depletion, industrialization, soil degradation,

Table 3: Standards for Air Quality Assessment (Levy and Marans 2012)

Air Quality standards Reference			
Pollutants	Average time	Method	Principle of detection
Sulpher Dioxide	3 hours or 24 hours	Pararosaniline	Colorimetric
Carbon Monoxide	1 hour or 8 hours	Spectrometry	Infrared
Hydrocarbons	3 hours	Gas chromatographic	Flame ionization
Ozone	1 hour	Spectrometry gas-phase O_3-ethylene	Chemiluminescence
Nitrogen dioxide	Annual	24 hours integrated samples in alkaline solution.	Colorimetric

Table 4: Contaminants measurements (Levy and Marans 2012)

Particle sizing and associated instruments		
Property measured	Method	Size Application(mm)
Length	Optical microscopy,	0.5-2000
	scanning electron microscopy, Transmission of electron	0.02-500
		0.002-10
Volume	Electrical resistivity	0.5-400
Mass	Sedimentation, Centrifuge, impaction	0.5-200
Surface area	Gas adsorption	0.001 m^2/g and up
Crystallite volume	X-ray diffraction	0.015-0.050

deforestation, urbanization and modification in the temperature cycle are anthropogenic. All these human activities have influential effects on the environment. Process of continuous natural environmental modifications, the transformation of natural resources into profitable artificial resources leading towards health hazards (Bashir et al. 2020).

Anthropogenic Effects

Global climate changes: The modification in the environment has a more significant impact on climate change. Extreme weather conditions, glacier melting, ozone depletion, increase in the amount and number of gases and UV radiation due to chlorofluorocarbon threatens human health and enhances the progression of diseases in human (Lan et al. 2012)

Invasion of exotic species: Manmade activities near the coastal line are directly associated with fisheries and marine life. The production of commercial fisheries results in the changes in environment of coastal life. The production of marine food at the commercial level leads to diminishing genetic diversity and extrinsic fertilization also deteriorates marine life (Rathore and Nollet 2012).

Eutrophication: The structural changes in the ecosystem due to the enrichment of water by nutrient salts and other pollutants is called eutrophication. Eutrophication is responsible to increases the algae, and aquatic plants, which are directly affected by fish species and marine food (Armeanu et al. 2018).

Use of fertilizers: Unethical practices in agricultural land cause the accumulation of pollutants, and chemicals in the environment. These pollutants are responsible for lung cancer, skin cancer, intestinal diseases, and congenital abnormalities (Asako 1979).

Accumulation of Wastewater into Drinking Water

Sewage and industrial wastewater are discharged into water reservoirs, or drinking water, which are responsible for viral, bacterial, and fungal infectious diseases in humans. Aluminum, phosphorous and heavy metals causes indigestion, stomach abnormalities, intestinal obstruction, and cancer (Dandachi et al. 2020).

Chemical hazards: Exposure to chemicals leads to the chronic disorders such as allergic reactions, high blood pressure, immune system disorders, nervous system disorders, and carcinogenic effects. Some chemicals used in industries such as methyl mercury, arsenic, lead, and toluene directly affected on fetal development and can cause mutagenic, teratogenic, and carcinogenic problems. Exposure to the chemical environment, acids, vapors, and fumes are considered as bomb exploding symbols for pregnant women. DNA mutation is responsible for congenital disorders, birth defects, early life malignancy, tumors, bone marrow cancer, and scrotal changes (Ader 1995). The causative environmental factors of various diseases have been described in Table 5 (Khopkar 2007).

Long-term exposure to chemicals even at the minimal level interrupted the nervous system, and act like a venom for human leading to loss of electrochemical message and behavioral changes occurred. Hormones are chemicals (natural) that are responsible for human behavior, internal body mechanisms, learning patterns, reproductive cycle, and certain cardiovascular, and respiratory rhythms. Hazardous chemicals disturb the endocrine functioning, normal hormonal patterns, cardiovascular disorder, and respiratory depression (James and Card 2012).

Biological Hazards: Biological substances are major health threats to humans involving healthcare workers, laboratory workers, contact with animals, exposure to pathogens, and infections. There are four groups under consideration of risk:

Table 5: Biological Contaminants and their causative Environmental factors

Diseases	Causative environment
Diarrheal diseases, hepatitis, intestinal worms	Basic sanitation of water, drainage, and sewage accumulation into drinking water.
Measles, Pertussis	Aerosols, overcrowding,
Dengue, yellow fever, Chagas disease, leishmaniasis.	Vector-borne, lack of sanitation, inappropriate personal hygiene
Eyes and skin infections	Water pollution, inappropriate sanitation
Tetanus	Accidental injuries
Respiratory tract disorders	Contaminated air (chemical, biological contaminants)

Group 1. Microbial entities, that are unlikely to cause diseases in humans.
Group 2. Microbial entities, that are likely to cause diseases in humans.
Group 3. Agents that cause various infectious diseases and hazardous to human health.
Group 4. Agents having serious pathogenicity with no available treatments (Vennemo et al. 2020).

Bioterrorism: The use and transmission of infectious agents in a concentrated urban population for mass-level pathogenicity and deaths are called bioterrorism. It is a cheaper and mass-level destructive biological weapon in comparison with nuclear weapons. COVID-19 is also considered a biological warfare (Kohtala and Hyysalo 2015). Many countries spent billions of dollars on the development and producing biological weapons. Recombinant DNA technology is used to develop biological agents (Dolk and Vrijheid 2003).

Physical Hazards: The release of energy in various forms that causes harm with or without contact is considered as physical hazard. It includes:

i) **Noise:** The most common and extreme physical hazard, that damages the ear drum, long-term exposure to noise leads to deafness. Noise is also responsible for cardiovascular disorders, hypertension, behavioral changes, and the reduction of erythrocytes in the serum. (Kassinis et al. 2016)

ii) **Vibration:** In industrial cities, modern machinery produces vibrations at a larger scale, which is the biggest cause of workplace stressors that affects the whole body. (Watson et al. 2012).

iii) **Pressure:** Above and below atmospheric pressure pose health hazards which is associated with certain occupation such as diving, aviation, etc. High altitude is affiliated with decompression and high-altitude sickness.

iv) **Radiations:** Electromagnetic radiation causes severe tissue damage at a larger scale. It is also a major contributor to cancer and gene mutation. (Little et al. 2016).

Extreme temperature: Working under very high or freezing temperatures causes depression in the body's normal functioning, which leads to pathological conditions (Saxena et al. 2003).

Global Warming and Human Health

Global warming causes climate changes which rise the floods, famines, extreme heat waves, and de-freezing of glaciers. The earth's biosphere releases and absorbs the gases like carbon dioxide, methane, and nitrogen dioxide which are significant parts of the atmosphere and weather. Anthropogenic activities modify these biogenic greenhouse gases and release methane in higher quantities. The release of chlorofluorocarbons depletes the ozone layer and is responsible for the greenhouse effect. The greenhouse gasses affect the atmospheric temperature and climate change. It includes CO_2, nitrous oxide, water vapors, chlorofluorocarbons, methane, and other trace elements. Causes of global warming include fossil fuel burning, coal, Petroleum, natural gases, deforestation and use of fertilizer (Shore and Shemesh 2016).

Effects of global warming: Heat waves produce extreme dehydration and death in older adults with respiratory and cardiovascular disorders. Heat stroke, pulmonary malfunctioning, cardiovascular collapse, allergic reaction, and phlebitis occur during the heat wave (Kim et al. 2021).

Control and Prevention of Air Pollution

Air pollution control should be considered under a total technology control system and environmental control system. There should be some important measures at the international level to prevent further damage to the environment and reduction of air pollutants (Hueting 2010). Following measures need to be taken to reduce air pollution:

- ✓ The prohibition of open burning or single chamber incineration.
- ✓ Low emission from transportation by using electric transport or less use of fuel-based transportation (O'Neill et al. 2003).
- ✓ Air control rules and regulations on the application of zoning ordinances that cause the sources of air pollution to be located or distributed to minimize the effect of air pollution.
- ✓ Imposition areal limits on emission rates that have been driven by air quality standard requirements (Seinfeld 1989).
- ✓ Air pollution zoning can be legally applied to prohibit new industries, expand on existing industries, and liquidate non-conforming industries and harmful industries (Jeswani et al. 2020).

- ✓ Reduce the hydrocarbons/lead emission from vehicle's by converting fuel into gaseous fuels i) Liquid natural gas (LNG) ii) liquid petroleum gas (LPG). It will reduce the particulates in the air.
- ✓ Improvement in operational practices such as food processing, storage of raw material, good sanitation, drying of residuals, grinding, minimization of local heating, and modification of incinerators into multi-chambered incinerators (Osbaldiston and Schott 2012).
- ✓ Gravity settling chambers/ centrifuge separators in industries to prevent particulates pollution (Riffat et al. 2016)
- ✓ The adsorption process can reduce the gas elimination in the air directly. Certain adsorbant can be used such as activated carbon adsorb vapors of gases. Benzene adsorbed by Hexanol.
- ✓ Condensation is another process of liquefying the gases.

Waste management and waste reduction are also useful to control contaminants (Stern 2018).

Smog

Smog is the combination of smoke and fog, which was coined in 1908 in United Kingdom. The word smog is a portmanteau of the two words. Fog is a visible aerosol which consists of water droplets, ice crystals and pollutants suspended in the air. It is also considered as low-lying clouds (Mishra 2017). There are multiple contributing factors, includes temperature, wind, sunlight, and pollutant gases. The chemical reaction formed in 1952, the city London experienced longer episode of smog, which was called killer smog (Briggs 2003). There was increased morbidity and mortality reported during that outbreak in 1952. The combustion of coal produces smoke than combines and causes deleterious health issues. In Pakistan, Bangladesh and India free smoke ejected in the air by the combustion process of coal and burning of fossil fuels in last two decades. The air quality comprised and caused health problems. It was observed that, particulates level and size increases to 10 µm in diameter to 3000 µg/ m3. In 1952 it was 30 µg/ m3 andin 2002 it became double. (Tilman and Clark 2014) The blanket of smog in industrial cities and agriculture land surrounded from last decade in winters. Chemical reactions occur between key pollutants in the air (Ali et al. 2019). The excessive pollutants are volatile organic compounds and nitrogen oxides which interact with hydroxyl, ozone, and nitrates. The main sources of these pollutants are emission coal fire power plants, traffic emissions, burning of fossil fuels and eruption of volcanoes. These activities directly or indirectly contribute to the production of PM 2.5 which is main precursor for smog production. Globally, the occurrence of smog has been reported in many major cities around the world includes Lahore, London, Delhi, Mexico, Los Angeles, Tehran, and Beijing. The significance of smog and health effects of smog started to attract attention since 1952. Now it's a well-known pollution around the world but difficult to tackle the pollutants (Dupont et al. 2016).

Air pollution is one of the most significant environmental concerns in recent decades. The situation has been worsening in Lahore, the provisional capital of Punjab. Lahore has a 4 % annual growth rate of economic and Pakistan's largest and most populated cities. In the latest air quality index Lahore ranked one of the most polluted cities in the world, Lahore ranked 6th, Karachi ranked 16th with air quality index points 170 and 155 respectively.

Types of Smog and Constituents (Dong and Hauschild 2017)

1. Classical Smog (London type)

The composition of classical smog is the mixture of smoke, fog and sulfur dioxide, which act as reducing agent. Classical smog also called reducing smog. Commonly in winter season.

2. Photochemical (Los Angeles type)

Photochemical smog also called summer smog is the chemical reaction of sunlight, nitrogen oxides, and volatile organic compounds in the atmosphere. This reaction leaves airborne particles and ground level ozone. Photochemical smog dependent on primary pollutants and produces secondary pollutants such as peroxyacetyl nitrate, tropospheric ozone, and aldehydes.

Human health compromised due to smog. It causes eye irritation, skin irritation, allergic reactions, breathing disorders, asthma, COPD, skin cancer, and breathlessness. Although smog have temporary health hazards, but continuous exposure to smog leads to chronic disorders. (Ones and Dilchert 2012)

Noise Pollution

An unpleasant sound that disturbs physiological and psychological parameters is called noise. Noise affects with different ways on the people. Noise levels can be measured in decibels. The human ear can detect a range of voice pressure, or mechanical movement (0 dB to 140 dB). The vibration transmits the voice from different mediums. The 20th century was described as "The Century of Noise". Noise is considered an important stress factor in the urban population. There are two important properties of noise which are contributing to stress and deafness. Noise level of more than 60 dB affects the human eardrum and cochlea. Continuous exposure to loud voices disturbs humans physically and psychologically. It will also influence the quality of life and impairment of work performance.

Table 6: Levels of noise in different areas (Dong and Hauschild 2017)

Different Area's	Maximum	Minimum
Commercial	88	62
Residential	82	67
Quite Region	75	53
Industrial	83	68

Table 7: Different Equipment's noise (Olah et al. 2020)

Household Equipment	Noise (decibel)
Vacuum cleaner	70
Washing machine	78
Refrigerator	45
Normal conversation	67
Whisper	30
MP3 player max volume	105
Lawn mover	85-90
Commercial equipment	
Jet take-off	130
Train	100
Jackhammer	110
Thunderstorm	120
Boom	110-120
Heavy traffic	85
Motorcycles	95
Sirens	120

Health and safety regulations are more stringent worldwide to prevent noise pollution. Anthropogenic noise is almost an omnipresent environmental stressor. There are two main types of noise pollution:

1. Intermittent
2. Chronic / frequent (Scoy and Tjeerdema 2014)

Table 6 depict the minimum and maximum levels of noise at different areas and Table 7 shows the noise level of various household equipment's.

Effect of Noise on Human Health

Long-term exposure to environmental pollutants leads to physical, and mental disturbance which ultimately converts into chronic illness. Physical problems include:

1. **Cardiovascular disorders:** Continuous exposure to noise more than 60 dB causes hypertension and cardiovascular disorders.
2. **Endocrine responsiveness:** High-intensity noise raises adrenaline and norepinephrine, which increases the stressors in the body.
3. **Psychiatric disorders:** Exposure to noise changes the human normal responsive behavior. As stressor increases in the body, coping and challenges solving ability reduces.
4. **Tinnitus:** Loudness causes tinnitus in the human ear, which affects mental health capabilities.
5. Some industrial workers reported nausea, vomiting, and other physiological problems.
6. **Deafness:** High-intensity noise for long-term causes deafness.
7. **Sleep disturbance:** Noise reduces the sleep hours, relaxation and deep sleep is ultimately compromised
8. **Reduce work performance:** Industrial workers' performance reduces with longer exposure to vibration and noise (Lambert et al. 2012; Thangavel and Sridevi 2016).

How to Reduce Noise Pollution (Morelli 2011)

Noise pollution is unavoidable in cities, but it can be avoided in many places.

1. **Reduction of appliance noise:** Indoor noise including washing machines, dryers, vacuum cleaners, fans, and other electrical equipment produces a noisy environment. The reduction of equipment's noise will reduce overall noise pollution.
2. **Reduction of noise from media voice:** The loudness of television, mobile videos, radio, and video games voice should be reduced to control noise pollution.
3. **Repair old machinery:** old machinery produces high pitch and volume. Repairing may reduce the volume and pitch.
4. **Soundproof:** Insulating strategies may reduce noise pollution.
5. **Ear protection:** If noise is unavoidable, ear protection is necessary such as earplugs or earmuffs.
6. **Create more quiet time:** Try to regulate a noise-free environment.

Land /Soil Pollution

7. Heavy metal toxicology is associated with potential toxicity in human and ecosystem. It is identified that several correlating factors are responsible for soil contamination (Bini 2010). There are multiple sources of these contaminants, which are responsible for human toxicity including agrochemicals i.e., fertilizers, pesticides, inappropriate irrigation, surface runoff, mineral extraction, industrial waste disposal, waste sludge, livestock manures and atmospheric deposition (Gao et al. 2016).

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There are multiple disorders directly associated with contaminated land which include skin rash, corneal infection, gastrointestinal disturbance, Asthma and cancer. Soil contamination can be prevented sthrough proper measures such as law enforcement, industrial waste management, and restrict the usage of inappropriate fertilizers to reduce the soil contamination and its toxicity (Bini 2010).

Conclusion

Population explosion around the world is responsible for the development of toxic environment in the form of gases and industrial explosion. Minor toxic elements are mainly responsible for health hazards. Human, animals, and plants are massively affected by these pollutants. Its causes multiple diseases including depression, respiratory, hematological, cardiovascular and neuromuscular disorders. The misuse of land, inappropriate agricultural practices, and explosion of plastic are not only dangerous for human but also for aquatic life. Law enforcement, proper agricultural practices, industrial and urbanization control can reduce the environmental toxicity.

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Different Molds and their Toxins: As Contaminants in one Health Perspective

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INTRODUCTION

Mycotoxins are fungal metabolites that are formed in cereal crops and pose substantial risks to both human and animal health. These are very dangerous and unavoidable food pollutants (Amaike and Keller 2011). Mycotoxicoses are acute and chronic toxic diseases caused by mycotoxins. Their clinical characteristics, target organs, and prognosis rely on the mycotoxins inherent toxic properties, the amount and period of exposure, as well as the exposed person's overall health.

Aflatoxin, deoxynivalenol, ochratoxins, zearalenone, and fumonisins are common in underdeveloped nations and are generated by a variety of fungal species including *Penicillium verrucosum, Aspergillus flavus, Aspergillus ochraceus, Aspergillus parasiticus, Aspergillus nomius*, and *Fusarium* spp (Vila-Donat et al., 2018). These fungi infect barley, wheat, corn, peanuts, cotton, and a variety of other commodities, causing immunotoxicity, hepatotoxicity, nephrotoxicity, and teratogenicity in animals and humans (Amaike and Keller 2011; Abdallah et al., 2017). When there is high ambient temperature and moisture, fungal growth and mycotoxins are produced. Other variables that contribute to production risk include incorrect storage, shipping, harvesting, and processing techniques (Uegaki and Tsunoda, 2018). It has attracted international attention due to economic costs in terms of animal production, commerce, and human health. The globe is now dealing with a food safety crisis, and a number of studies have been undertaken to address this issue by informing consumers about food safety issues (Rebouças et al., 2017; Kwol et al., 2020).

In 1985, the US Food and Drug Administration (USFDA) established a diagnostic laboratory to monitor feed contamination with mycotoxins and assure a toxin-free diet. The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) play major roles to prevent contamination by providing consumer education. Mycotoxins cannot be destroyed via cooking. The only option to obtain mycotoxin-free food is to use modern food processing techniques such as Hazard Analysis Critical Control Points (HACCP) and good manufacturing processes (GMP) (Maldonado-Siman et al., 2014). When mycotoxins are formed in crops, they have a direct detrimental influence on farm animals and can cause major health problems in human beings (Yang et al., 2020).

Mycotoxicoses have historically been common in mild zones, and today they are mostly seen in tropical areas. The global danger of mycotoxicoses is increased by international trade in foods from tropical nations. Medical professionals should at least be aware of the clinical symptoms of the most widespread mycotoxicoses, even though it is unrealistic for them to be able to diagnose mycotoxicosis in a single patient. This chapter aims to provide background information on mycotoxicoses as well as a clinical overview of one health triad.

Mycotoxins' Effects on Animal Health

There are several forms of mycotoxins that have diverse pathogenic and immunological impacts on animal species. Listed below are the brief description of all pathogenic and immunological effects on human beings and all other animals.

Effects of Zearalenone on Animal Health

Fusarium (F.) molds (*Fusarium graminearum, F. culmorum, F. crookwellense*) cause damage to wheat, oat, sorghum, and barley and generate Zearalenone (ZEN). *F. graminearum* is the primary producer of ZEN. In agricultural animals (cattle and sheep), it possesses estrogenic action. Zearalenone and its metabolites (α-zearalenol) bind to the estrogen receptor and induce a range of reproductive issues due to their structural similarities to estradiol (McCauley et al., 2017). ZEN was related with feminization, decreased libido, lower blood testosterone levels, decreased testicular weight, and altered spermatogenesis in boars (Mirza Alizadeh et al., 2022). In pigs, ZEN poisoning caused vulvovaginitis, fetal mortality,

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anestrus, reduced LH and P4 production, delayed post-weaning estrus, and promoted germinal epithelial degeneration (Imran et al., 2020). Another study indicated that ZEN toxicity resulted in decreased conception rate and litter size, as well as hypertrophy of the vulva, uterus, and ovaries in piglets (Grenier et al., 2019). ZEN toxicity in cattle resulted in reduced milk output, infertility, elevated serum estrogen concentrations (Liu and Applegate 2020), and a lower conception rate. Reduced testosterone levels in blood and sperm counts were seen in rats (Sadeghi et al., 2020), but in mice, chronic estrous and sterility (Pócs 2019), genotoxicity, and hepatocellular cancer were discovered in ZEN toxicity (Ruan et al., 2022).

Effects of Ochratoxins on Animal Health

Molds of the *Aspergillus* and *Penicillium* species generated ochratoxin-A (OTA). The principal species that generated OTA were *Aspergillus (A.) ochraceous*, *A. sclerotiorum, A. melleus*, *A. sulphureus*, and *Penicillium (P.) verrucosum* (Han et al., 2018). Multiple foods are naturally contaminated with OTA, including cereals, almonds, cocoa beans, cassava flour, peanuts, dried fruits, chicken eggs, and milk (Kumar et al., 2018). In natural settings, 50% of cocoa beans and 22% of cocoa powder were found to be contaminated with OTA (Maciel et al., 2018). The toxicological effects of OTA varied amongst poultry species. However, it is nephrotoxic to all animals and it is most likely to be harmful to humans. OTA is also known to be hepatotoxic, neurotoxic, immunosuppressive, teratogen, and carcinogen (Zhu et al., 2017).

According to reports, OTA causes oxidative stress in human kidney-2 cells, which regulates the translocation of transcription factors such as the aryl hydrocarbon receptor (AhR) and the pregnant X receptor (PXR). By modulating cytochrome enzymes (CYP1A1 and CYP1A2), OTA activation of Ahr and PXR causes immunosuppression, renal damage, and malignancy (Zhang et al., 2022b). The first epidemics of ochratoxicosis were discovered in laying hens, broiler chickens, and turkey birds. Clinical symptoms reported in turkey include reduced feed intake, a low FCR, and a higher death rate. Nephritis and mild hepatitis were the pathological lesions. In broilers, there was a decrease in growth rate, a low FCR, nephrotoxicity, and air-sacculitis. Reduced egg production and nephritis were reported in laying chickens (Imran et al., 2020). In cases of ochratoxicosis, necropsy findings include swollen and pale-yellow kidneys, as well as a yellowish and friable liver. Vacuolar atrophy and megaleucocytosis of liver cells, biliary epithelial hyperplasia, and hypertrophy of proximal convoluted tubular epithelial cells of the kidney are among the microscopic abnormalities (Tahir et al., 2022). According to Kumar et al., (2020), OTA is more nephrotoxic than hepatotoxic in broilers. OTA also infects other organs, causing spleen, thymus, and bursa shrinkage as well as lymphocytic depletion. It has also been observed that OTA has a deleterious impact on bird performance, hematology, biochemistry, and the immune system in laying hens.

In poultry, OTA reduces the weight of immune organs (bursa and spleen), the quantity of antibodies (IgA, IgG, and IgM) carried by immune cells, and they react to phytohemagglutinin (PHAP) (Khan et al., 2017). It reduces hematological parameters (RBC count, WBC count, PCV, HB) and causes anemia in White Leghorn Cockerels. It lowers blood total proteins while increasing serum alanine transferase, urea, and creatinine (Orinya et al., 2016). There was no severe OTA health issues reported in cattle fed with naturally contaminated OTA feed. In one investigation, wheat contamination with OTA (390-540 g/kg) and AFB1 (12-13 g/kg) had no significant toxic-pathological impact in 12-week-old calves (Shida et al., 2022). Cattle may degrade up to 12 mg/kg body weight of OTA (Hashimoto et al., 2016). Calves die after being fed with 11-25 mg OTA/kg body weight orally. For cattle, the fatal dosage of OTA is greater than 13 mg/kg body weight when administered orally (Ricci et al., 2020). According to a study, it contaminated feed (48g/kg) causing feed refusal, reduced milk output, and retained fetal membrane in dairy cattle, however no signs or symptoms were detected in beef cattle. Ochratoxicosis is uncommon in small ruminants (sheep and goats). However, consuming an OTA-contaminated food over an extended period of time has negative health consequences (Malekinezhad et al., 2021). OTA typically affects the kidneys in dogs and cats. At the dose of 0.2 to 3 mg/kg, it can cause vomiting, anorexia, increased thirst, polyuria, ataxia, and death. Tubular epithelial degeneration, hemorrhagic enteritis of the large intestine, and necrosis of lymphoid organs (thymus, spleen, lymph nodes, and lymph nodes) are all pathological lesions (Gan et al., 2017).

Effects of Aflatoxins on Animal Health

Aflatoxins are poisonous substances produced by certain *Aspergillus (A.)* species (*A. flavus, A. parasiticus, A. nomius, A. arachidicola, A. bombycis, A. pseudotamarii, A. minisclerotigenes, A. rambellii*), which are found all over the world and pose serious health issues in animals (Pereyra et al., 2020). Aflatoxins are classified as B1, B2 (generates blue fluorescence) and G1, G2 (emits green fluorescence) (emits green fluorescence). The liver is the principal target of aflatoxin B1 (AFB1) vulnerable animal species. The metabolic activation of AFB1 is strongly correlated with Cytochrome p450, which are expressed in the liver. This CYP converts aflatoxin to a reactive 8, 9-epoxide that can bind to DNA and protein. AFB1-DNA adducts cause G to T trans version in the tumor suppressing gene p53, which is linked to liver cancer (Mungamuri and Mavuduru 2020). Pyrexia, anorexia, and malaise are early signs and symptoms of aflatoxicosis, and as the disease progresses, the animal has stomach discomfort, vomiting, and hepatomegaly. Acute aflatoxicosis is less common (Al-Ruwaili et al., 2018; Kyei et al., 2020), but it has carcinogenic and immunosuppressive consequences in the chronic stage (Qian et al., 2016).

AFB1 reduces the production of IL-4, which has anti-inflammatory properties, and promotes the release of IFN-κB p65 and TNF-α from natural killer (NK) cells, which speeds up the inflammatory process. AFB1's immunosuppressive function is due to a decrease in the ability of antigen presentation cells (APC) of dendritic cells (Mehrzad et al., 2017). AFB1 causes more cancer episodes than the others (Shahba et al., 2021).

Aflatoxin has a harmful and cancerous effect on both animals and humans. The acute poisoning results in mortality (Martins et al., 2018). Chronicity is characterized by immunosuppression, the development of neoplasms, and a variety of other clinical disorders. In birds, fish, nonhuman primates, and rodents, AFB1 causes necrosis and degeneration of the hepatic parenchyma. The frequency of aflatoxicosis is determined by a variety of factors, including the animal's breed, species, age, diet, and overall health (Alvarado et al., 2017). Broilers are much more resistant to aflatoxicosis than some other poultry birds (Kraieski et al., 2017). AFB1 is also known as the "silent killer" since it is an inevitable pollutant whose continuous pollution causes toxicopathological consequences. Acute aflatoxin poisoning results in decreased feed intake, decreased feed conversion ratio, changed natural behavior, immunodeficiency, hepatomegaly, nephritis, altered hematological parameters, and overall impaired performance. The chronic aflatoxicosis has been linked to increased blood urea nitrogen (BUN), reduced total protein and lipid levels in broiler bird serum (Rashidi et al., 2020). It exhibits toxicological effects in animals, causing a variety of outcomes such as acute liver injury, liver cirrhosis, tumor induction, teratogenicity, and other genetic abnormalities. Aflatoxicosis is mostly a liver disorder (Afum et al., 2016) causing gastrointestinal problems, reduced milk supply, immunosuppression, anemia, and liver damage. AFB1 conversion to its metabolite AFM1 secreted by dairy cows in milk makes nursing animals more susceptible to infection (Frazzoli et al., 2017). Many researches on the carcinogenic effects of AFB1, AFM1, and AFG1 in various animals have been undertaken (Yilmaz and Bag 2022).

Sheep have a higher resistance potential to aflatoxins (Cammilleri et al., 2019). According to a research, AF-contaminated feed (2.5 mg/kg) causes hepatotoxicity, nephritis, altered mineral metabolism, and a reduction in plasma mineral (Vila-Donat et al., 2018). Another research with the same dosage results in increased fibrinogen concentration (Rastogi et al., 2022). Alvarado et al., (2017) discovered that AFB1 contaminated feed (58.4 g/kg) caused hepatomegaly, nephritis, and bile duct hyperplasia in horses. Depression, jaundice, lameness, and mortality were the most common clinical indications of aflatoxicosis. Other symptoms of horse aflatoxicosis include subcutaneous bleeding, intestinal lesion, kidney problems, liver necrosis, and cardiac lesions (Kemboi et al., 2020).

Effects of Fumonisins on Animal Health

Fumonisin B1 and Fumonisin B2 are carcinogenic metabolites of *Fusarium (F.) verticillioides* and *F. proliferatum* (Nassif et al., 2018). Because of its neoplastic activity in rats, fumonisin B1 is more hazardous than fumonisin B2, and it also induces horse leukoencephalomalacia (Rehman et al., 2022). Fumonisin inhibits the enzyme sphinganine-N-acetyl transferase, which is involved in sphingolipid metabolism, causing an increase in sphinganine and sphingosine as well as a decrease in sphingolipid complex, which is a widely acknowledged explanation for fumonisin toxicity in most species (Abdel-Wahhab et al., 2018). Fumonisin toxin has been shown to impact several organs in chickens, calves, pigs and horses, including the intestines, brain, liver, and lungs (Chen et al., 2021).

It causes pathological lesions on the liver and other internal organs, swollen beaks, cerebral edema (Chen et al., 2016) and hemorrhages in the subcutaneous and hepatic parenchyma (Rodríguez-Bertos et al., 2020). Clinical signs that are dose-dependent in broiler chicks include slower weight gain (Saleemi et al., 2020), but in layer birds it causes diarrhea (Dadheech et al., 2016). In equines it causes leuko-encephalomalacia, malnutrition, incoordination, lungs and brain edema (Wangia 2020), liquefactive necrosis of white matter, cerebral cortical lesions, hypersensitivity, and death (Junker et al., 2020). Fumonisin caused liver and renal inflammation in ovine at doses ranging from 11.1-45.5 mg/kg body weight (Cimbalo et al., 2020). Fusarium mycotoxins impact cow reproductive activities by altering granulosa cell growth and steroid synthesis (Albonico et al., 2017).

Mycotoxins and Human Health

Mycotoxicosis occurs in humans as a result of eating fungal-contaminated food, and it can be acute or chronic, depending on the length of the condition (Anal et al., 2021). *Fusarium* species were implicated in multiple human epidemics of mycotoxicosis prior to the discovery and application of current milling procedures. Between 1932 and 1947, Russians suffered from alimentary toxic-alleluia, which was characterized by mucus membrane hyperemia, esophageal discomfort, inflamed larynx, trouble breathing, GIT inflammation, and ataxia. This was attributable to the consumption of infected cereal grains with *F. sporitrichoides* and *F. poae* (Milićević et al., 2016).

Aflatoxicosis mostly affects the liver, resulting in hepatitis and jaundice, which can lead to death. In Kenya, India, and Malaysia, several episodes of the same kind of aflatoxicosis (hepatitis) were detected in various years (Kamala et al., 2018). AFB1 has been related to liver cancer in conjunction with the hepatitis B virus and is a human carcinogen (Rindi et al., 2018). There have been several reports of AFB1 being linked to hepatocellular cancer in conjunction with HBV infection (Chu et al., 2017). This is because of the prolonged exposure to a low dosage of AFB1. In China, around 250,000 individuals died from liver cancer, and further analysis found that the consumption of AFB1 was a contributing factor to HBV infection (Kumi et al., 2016). Ochratoxin-A causes nephropathy and renal carcinoma in animals, but its consequences on human health are not well understood. Research revealed that OTA contaminated feed was linked to human disorders such as chronic interstitial nephritis (CIN), Balkan endemic nephropathy (BEN), and other renal malformations, however the mechanism has not been thoroughly investigated (Stiborová et al., 2016).

Consumption of fumonisin-contaminated sorghum and maize in India caused food poisoning with stomach discomfort, borborygmi, and diarrhea (Achar and Sreenivasa 2021). In humans, IARC categorized fumonisin B1 as a Group 2B carcinogen (Organization 2018). Fumonisin poisoning has also been linked to lower folic acid absorption and neural tube developmental abnormalities in rural populations who fed contaminated maize in specific areas of North China and South Africa (van Gool et al., 2018). Fumonisin toxicity has also been connected to esophageal cancer in China as a result of fumonisin-contaminated feed intake (Chen et al., 2018). Trichothecenes have also been employed as a biological agent. T-2 toxin was employed as germ warfare in 'yellow rain' against the populace of the Democratic Republic of the Congo from 1975 to 1981 (Jung 2016). Similar biological warfare chemicals, such as DAS, nivalenol, DON, and ZEN, were identified from water sources and leaves in afflicted areas of Cambodia (Anal et al., 2021). Toxicity from zearalenone (ZEN) and deoxynivalenol (DON) have been recorded in the United States, Australia and Japan, leading to gastrointestinal symptoms such as nausea, vomiting, and diarrhea. Zearalenone has also been shown to be hazardous to human and animal gametogenesis and embryo development (Liu et al., 2018).

Occurrence and Toxicity of Zearalenone

Fusarium fungi produced zearalenone mycotoxins, which contaminates cereals and food stuff throughout the world. ZAE shows reproductive disorders in laboratory animals and also cause low acute toxicity. ZEA mostly occurred in cereal crops and foodstuffs around the world including Europe, Asia and Africa (Rai et al., 2020).

ZEA toxicity causes a limited effects on human health, but it can cause hyper estrogenic syndrome in humans. According to studies, high level of ZEA present in serum of children who were suffering in idiopathic puberty. ZEA could be the possible cause of early puberty in children as maize and their products are more prone to ZEA (Rai et al., 2020). It can also affect endometrium of women having reproductive disorders, leading to estrogenic effects. Occurrence of esophageal and breast cancer has also been reported from different parts of the world (Belhassen et al., 2015). Fig. 1 illustrates the absorption, metabolism and distribution of Zearalenone in different parts of the body.

Fumonisin

Fumonisin is produced from *Fusarium verticillioides*, and fumonisin B1 is mostly found in nature (Robert et al., 2017). According to human Epidemiological studies, esophageal cancer could be due to the consumption of corn grains which contains fumonisin in South Africa, Italy and China (Braun and Wink 2018).

Fumonisins are competitive inhibitors of sphinganine and sphingosine N-acyltransferase. It inhibits enzymes which produces a disruption of sphingolipid metabolism. It increases sphinganine and sphingosine and decreases complex sphingolipids in the serum and tissues of animals (Pierron et al., 2016). It can cause diarrhea and abdominal pain if the intoxication is acute and their chronic exposure can cause esophageal cancer (Smith 2018).

The exposure of fumonisin has also been linked with neural tube defect in many species including humans. Due to the inhibition of the fumonisin enzyme, this action is strongly related (Jin et al., 2021).

Aflatoxin

Aspergillus flavus, *A. parasiticus* and *A. nomius* produce secondary metabolites called aflatoxin which is highly toxic. Mostly wheat, walnut, peanuts and tree nuts are infected by these fungi. It can cause serious issues to human and animal health by causing various complications like hepatotoxicity, teratogenicity and immunotoxicity (Kumar et al., 2017).

Aflatoxins can cause damage at molecular level such as DNA mutation, posttranslational peptide chains modification, proteins and nucleic acids methylation and formation of free radicals. Aflatoxins have cancerous effects and occur frequently in food, that’s why these are continuously examined in some groceries and agricultural products (Kowalska et al., 2017).

According to recent evidence, aflatoxins can be determinant of stunted child growth and lower cell mediated immunity, that’s why increases disease susceptibility. However, since this is only a temporary relationship, more research is needed (Gong et al., 2016).

Ochratoxin

Ochratoxin is produced by different fungi including *Aspergillus Ochraceus, A. carbonarius, A. Niger* and *Pencillium verrucosum*. It is found in large variety of agricultural products such as cereal grains, dried fruits, wine and coffee (Ropejko and Twarużek 2019). OTA involve in the pathogenesis of some renal diseases like Balkan endemic nephropathy, kidney tumors occurring in Balkan Peninsula. Chronic interstitial nephropathy occurs in Tunisia and other countries in North Africa (Malir et al., 2016).

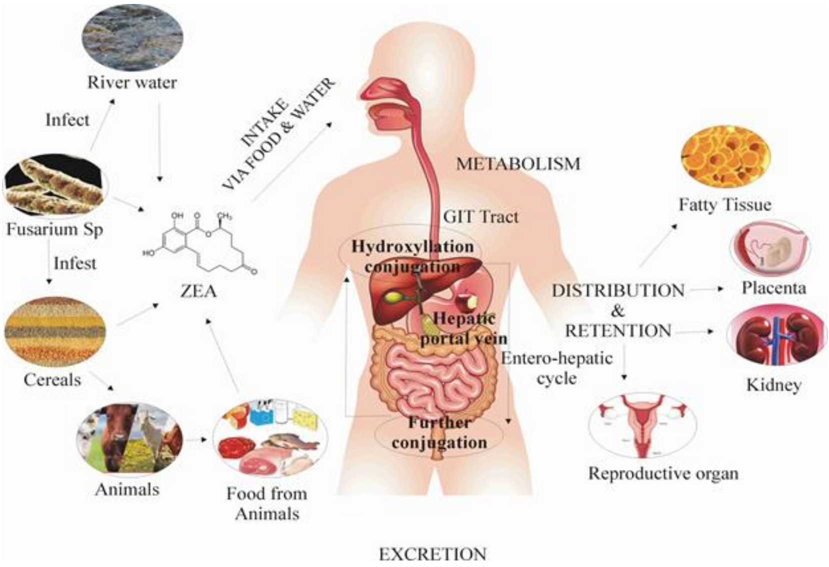

Fig. 1: Toxico-kinetics of Zearalenone (ZEA): absorption, metabolism and distribution in different body parts.

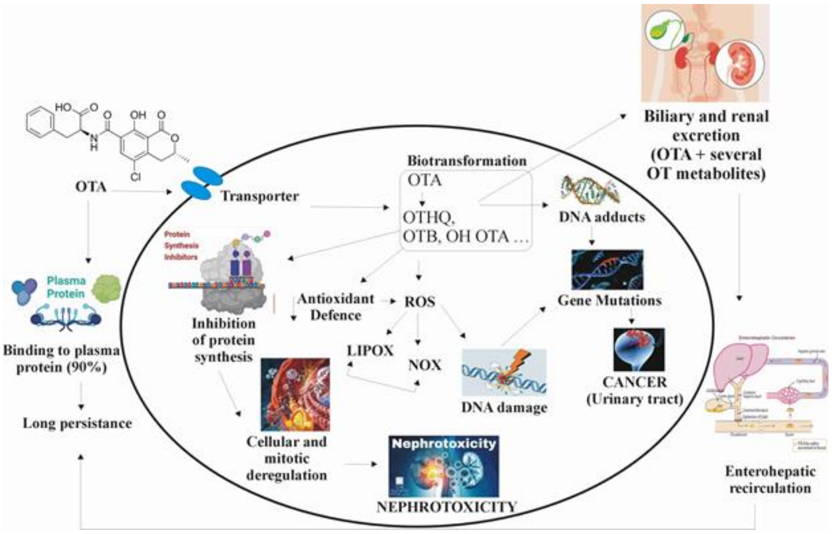

Fig. 2: Diagrammatic representations of Ochratoxin A (OTA) pathway and intracellular pathological effects. OTHQ: Hydroxyl Quinone Ochratoxin OTB: Dechlorinated ochratoxin LIPOX: Lipoperoxidation OH: *hydroxyl* free radical ROS: Reactive oxygen species, NOX: Nitrogen oxide.

In animals, ochratoxins involved in detrimental effects, such as renal, neuronal, immuno and embryonic toxicity. There may be unexplored fungus species where the occurrence of OTA may be attributed (Kortei et al., 2021). Fig. 2 shows the diagrammatic representations of Ochratoxin A (OTA) pathway and its intracellular pathological effects.

Impact of Mycotoxins in the Environment

Globally, there is a greater focus on air pollution. Mold is one such contaminant that may be found not just outside but also inside environments with excessive dampness. Mycotoxicosis is a multidisciplinary problem that requires the participation of environmentalists, medical and veterinary professionals, agricultural engineers, plant pathologists, analytical chemists, and agronomists. It occurs in both developed and developing nations when social, environmental, and economic factors combine with climatic conditions (temperature and humidity) that encourage mold development (Dragan et al., 2019).

Mycotoxins and agricultural product contamination fluctuate from year to year, based on the environment and handling circumstances. Multiple research projects have been conducted to investigate the negative effects of fungus-contaminated environments, particularly *fusarium*, on the respiratory and cutaneous systems. Ochratoxins (A, B, and C) and trichothecenes (T-2) are two significant classes of mycotoxins that have been detected in food and water-damaged structures and have been linked to substantial health problems in humans (Ratnaseelan et al., 2018). It has been claimed that trichothecenes can be produced at 300-fold the concentration of spores normally seen in the air of polluted buildings (Awuchi et al., 2021), causing multisystem effects in those who are exposed (Ratnaseelan et al., 2018).

Climate change has a substantial impact on food security, such as toxigenic fungus infection and contamination with mycotoxins. Acclimatization of mycotoxigenic fungus and synthesis of mycotoxins, particularly AFB1, appears unaffected by climate change, leading to ongoing contamination of animal feed and human food, culminating in major health problems in animals and humans (Sadiq et al., 2019).

Major Environmental Mycotoxin Sources

Climate change is mostly responsible for the AFB1 or ZEN contamination of maize and wheat harvests (Gruber-Dorninger et al., 2019). The amount of mycotoxins in the soil is determined, how they get there, whether directly or through contaminated straw (Zhang et al., 2022a). Additionally, after runoff or rainfall, mycotoxins will move from agricultural areas to surface waters (Juraschek et al., 2022).

Crops that are contaminated by mycotoxins are turned into feed, which is subsequently consumed by animals or humans and discharged into the environment after digestion. Its contamination persists in animal and human excreta, resulting in a substantial source of secondary environmental pollution (Zhang et al., 2022a).

About 50% of Aflatoxin B1, 20% of ZEN and 37% of DON will be expelled in feces, according to sampling evidence studies done to determine the level of mycotoxins in animal feces (Zhang et al., 2022a).

Aflatoxin

In nature, there are more than 20 different forms of aflatoxins including their derivatives, but only four, types namely B1, B2, G1, and G2, have been shown to be harmful to both humans and animals. The main Aspergillus strains that generate AFs, or furanocoumarins, are *A. flavus*, *A. parasiticus*, *A. nomius*, and *A. pseudotamarii* (Ráduly et al., 2020).The most cancer-causing and thoroughly researched AF is aflatoxin B1 (Benkerroum 2020).

AFM1 is a 4-hydroxy derivative of AFB1 that is produced in the liver and secreted into milk by human and nursing animal mammary glands that have consumed AFB1-contaminated food. AFB1 is converted into aflatoxin B1-8,9-epoxide (AFB0), which has both exo and endo isomer, in the liver via the cytochrome P450 enzyme system (CYPs) (Cao et al., 2022).

Acute aflatoxicosis causes mortality, however chronic exposure causes cancer, immunosuppression and pathological problems that take time to show. Chronic aflatoxin exposure reduces DNA replication in the bone marrow, which results in low cell counts (Benkerroum 2020).

Ochratoxin

Mycotoxins are toxic secondary metabolites produced by some fungal species. As per reports, more than hundreds of mycotoxins have been discovered so far (Gizachew et al., 2019).

Ochratoxin A, Ochratoxin B, and Ochratoxin C are the three varieties of ochratoxins that are classified based on their chemical makeup (Wang et al., 2022). It is hard to avoid ochratoxin because it is present in many dietary products. Studies show that ochratoxins do not pose a serious harm to the general public's health. The most common species in food are *A. ochraceus*, mesophilic xerophile species *A. styenii*, and *A. westerdijkiae*, which can be dangerous before and after harvest in cereals, fruits, coffee, cocoa beans, and nuts. Ochratoxin is primarily produced by *A. chraceus*, which can grow in environments with temperatures between 8 and 40 degrees Celsius, high water activity (aw) values between 0.9 and 99 and pH levels between 3 and 10 (Gizachew et al., 2019).

A. ochraceus can also produce toxic metabolites like penicillic acid, viomellein, vioxanthin and xanthomegnin. Ochratoxin found in many tropic and sub-tropic foods such as fresh fruits, dried fruit wine and some vegetables (Wang et al., 2022).

Animals can be infected through feed and humans through beverages and food. Ochratoxin enters in the circulatory system through gastrointestinal system. It has higher affinity with serum albumin and proteins so it accumulate in organs of human and animal (Yang et al., 2022).

Fumonosins

Aflatoxin, ochratoxin, zearalenone, and trichothecene mycotoxins are of lesser concern than fumonisin because of their frequent presence and possible harm to human and animal health (Wangia-Dixon and Nishimwe 2020).

Abalone fed with fumonisin-contaminated feed demonstrated that the toxin leached into the saltwater and affected the aquatic ecosystem. Fumonosins are formed on relatively common seaweed abalone (Greeff-Laubscher et al., 2020).

The major source of fumonisin production is the gene cluster fumonisin biosynthetic gene (FUM) in Fusarium and Aspergillus. The expression of these genes is co-regulated, and the expression of the FUM genes is connected to them as well (Pierron et al., 2016).

Not only the crops, but many popular ornamental plants e.g., aster begonia, carnation, chrysanthemum, gladiolus, etc. are mostly attacked by different *Fusarium* species, viz., *F. oxysporum*, *F. foetens*, *F. hostae*, and *F. redolens* at different stages of production (Sokolovic et al., 2022).

Table 1: Toxic effect different toxin on one health triad

Type of Toxin	Study Model/ Animal Type	Dose	Results	Overall effect	Reference
AFB1	Kunming mice	450 µg/kg BW	↑Gene expression for oxidative stress and apoptosis	Oxidative damage Apoptosis	(Li et al., 2021a)
AF	Animals		↓ Feed intake	Milk production Egg production	(Sarma et al., 2017)
AF	broiler chicks	50 and 100 ppb	↑ ALT ↑ ALP ↑ AST	bile-duct hyperplasia periportal fibrosis hydropic degeneration fatty change	(Ortatatli et al., 2005)
ZEN	E. coli	23.0 U/mg, 64.7 U/mg, 119.8 U/mg and 66.5 U/mg	↓ Enzymes activity ↓ Bacterial growth	Detoxifying agent in food and feed industry	(Wang et al., 2018)
ZE	In Vitro and In Vivo Studies	40 µM	PI3K/Akt-Mediated Nrf2 Signaling Pathway	Oxidative Stress and Apoptosis	(Rajendran et al., 2021)
AF	Rats	2 mg/kg body wt.	↓ Mortality, body weights, feed intake, and litter weights ↓ Embryonic resorptions and fetal body weights	maternal and Developmental toxicity	(Mayura et al., 1998)
AF	Broiler	211.88 µg / kg	↑ Vacuolar degeneration of hepatocytes, ↑ thickening in the wall of bile duct associated with leucocytic cells infiltration in the portal triad ↑ Necrobiotic changes of renal tubular epithelium with pyknosis of their nuclei and hypercllularity of glomerular tufts. ↑ necrosis of neurons ↑ neuronophagia of necrotic neurons and necrosis of purkinje cells of the cerebellum		(Shebl et al., 2010)
Fumonisin	Escherichia coli	10 µg/ml	↓ detoxification enzymes		(Li et al., 2021b)
ZEN, OTA, FB1, AFB1	Experimental animal	0.5 µg/kg bw/day 100–120 ng/kgbw/day 2 µg/kg bw/day -	↑ Changes Microbiota composition ↑ Changes metabolic activity ↓ Growth ↑ Immunotoxicity		(Robert et al., 2017)
OTA	broiler chicken	50 µg/kg	↓ Immune response ↑ Depression in phagocytosis		(Casafranca Loayza 2018)
OTA	Lab animals	100–120 ng/kg bw/day	↑ Nephrotoxic (renal tumors) ↑ Carcinogenic		(Robert et al., 2017)
OTA	albino rats	50 ppb OTA and 25 ppb ZnONPs	degeneration, ↑ sinusoidal spaces ↑ infiltration of lymphocytes ↑ vascular congestion and necrosis.		(Hassan et al., 2021)
ZEN	Bacillus	1.20 ± 0.11, 0.47 ± 0.22 mgkg1	Degrade ZEN in feed and alleviate the adverse effect of ZEN for piglets.		(Wu et al., 2021)
ZEN	Pigs	1.0 ppm	hypererestrogenic syndromes		(Rogowska et al., 2019)

↑: increase ↓: decrease AFB: Aflatoxins B ZEN: Zearalenone OTA: Ochratoxin A FB1: fumonisin B1 ALT: alanine transaminase ALP: *alkaline phosphatase* AST: Aspartate Aminotransferase

Zearalenone

When zearalenone (ZEN) (a substantial family of mycotoxins that is classified as a xenoestrogen) binds to estrogen receptors in a manner similar to that of natural estrogens, it causes a variety of reproductive problems, including hormonal imbalance (Sohrabi et al., 2022).

Zearalenone, a mycotoxin produced by fungi of the genus *Fusarium*, widely exists in animal and human food (Han et al., 2022). Because of its toxicity and estrogenic action, zearalenone can grow in fresh flowing water, disrupting the aquatic ecology and endangering both human and aquatic biota (Kortei et al., 2021). Zearalenone mycotoxins, which inflict catastrophic effects on the organs and remain in the tissues of animals, will be produced in animal feed because of the inadequate storage conditions. Humans will ultimately ingest these wastes causing serious complications (Zhang et al., 2022a). Zearalenone is highly toxic and can affect the health of animal and humans. It also provides insight to its harmful effects on human health and agriculture along with its effective detection, management, and control strategies (Mahato et al., 2021). Fig. 3 shows the schematic diagram illustrating the effects of different toxins on one health triad. Table 1 shows the effects of different toxins on human, animal, and environmental health.

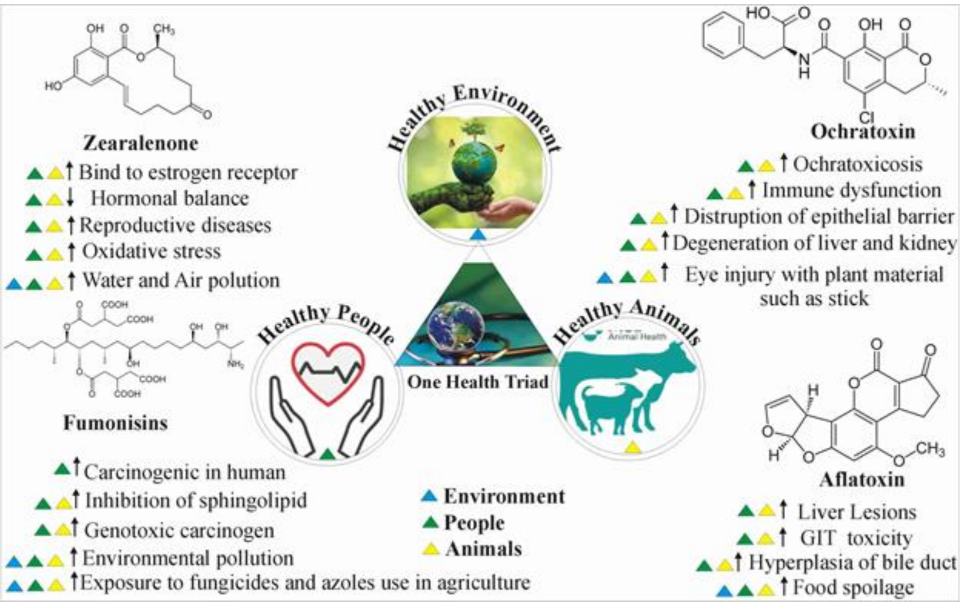

Fig. 3: Schematic diagram illustrating the effects of different toxins on one health triad i.e. Effect of ochratoxin (Hassan et al., 2021), zearalenone (Gao et al., 2022), Fumonisin (Pierron et al., 2016) and Aflatoxin (Qazi and Fayyaz, 2006) on humans, animals and environment.

Conclusion

Mycotoxicosis is a worldwide food safety concern. The FAO and WHO expert committees examined the risk analysis of mycotoxins toxicity and concluded that it is a major concern in developing countries since they can cause serious and irreversible health problems in animals and humans. Meanwhile, the possibility cannot be ruled out that environmental and biological variables influence the presence and prevalence of fungal pathogens in stored agricultural products (Sarwar et al., 2018).

It is well understood that once mycotoxins have contaminated feed or food, nearly nothing can be done to eliminate them. The most fundamental and effective method for reducing fungal infection at sensitive agricultural storage facilities is to manipulate environmental conditions. This will reduce the entry of fungal-derived mycotoxins into the feed and food chain, ultimately reducing their negative impacts on animal and human health. We can address the shortcomings of this global one-health issue by enlisting multidisciplinary professionals and relevant authorities to play critical roles in risk factor assessment and mitigation, research gap identification, basic and applied research promotion, public awareness, and capacity building.

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Mycotoxins Incidence in Animal Feeds, Their Prevention and Control Measures

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INTRODUCTION

The demand for livestock production and their products is the main prospect of livestock feeds globally. This increase in demand of animal feed is the result of increasing growth of world population. Food and Agriculture Organization evaluates that need for foodstuff will rise up to 60% by 2050, and protein production from animal origin will increase by 1.7% /year from 2010-50, with beef production likely to increase by nearly 70%, aquaculture by 90%, and diary by 55% (IFIF 2021). Mycotoxins are toxic secondary metabolites produced by molds naturally. Some mycotoxins like aflatoxins (AFs), fumonisins (Fms), ochratoxins (OT), trichothecene, and. zearalenone are predominant in animal feedstuffs. More than 500 either regulated or tested mycotoxins have been identified (Awuchi et al. 2021) causing diseases to living organisms. Three fungal genera: Aspergillus, Penicillium and Fusarium produce mcotoxins (Bennett and Klich 2003).

Mycotoxin formation generally occurs in the field, during processing and storage of feeds under unfavorable conditions. Fungal colonization, water activity and ambient temperature are major factors for mycotoxin production (D'mello 2002). Some drawbacks with intake of contaminated feed in livestock are low feed consumption, poor feed conversion, reduced body weight, greater syndrome prevalence and reduced reproductive capacities (Gashaw 2016), causing large costs on economy (CAST 2003).

Nevertheless, it is very important to understand some potential sources of mycotoxins that contaminate livestock feeds.

Classification of Fungi and Mycotoxins

Mycotoxin formation can occur at pre and post-harvest stages. Based on these two stages and climatic conditions, classification of toxigenic fungi is summarized in Table 1. It is noteworthy that only following four classes of toxigenic fungi cause mycotoxins formation. A summary of various mycotoxins caused by different fungi in several feed and foodstuffs along with their toxigenic effects at different biological activities are depicted in Table 2.

Dynamics of Fungal Growth and Mycotoxins Formation

Fungal colonization and subsequently mycotoxin production are generally affected by variable determinants. Some stress factors such as drought, poor fertilization, high crop densities, weed competition and mechanical damage can weaken the plant's natural defense system and promote mycotoxin formation. Optimal temperature and water activity for mycotoxin production range between 20 to 30°C and 0.9 to 0.99aw, respectively (Table 3). Water activity, oxygen content, and surface area are the physical characteristics of substrate, while chemical features including availability of different nutrients such as starches, oil and fats, vitamins, and amino acid composition cause mycotoxin production (Zaki et al 2012).

Storage fungi can grow at a minimum water activity of 0.70 aw; however, water activity for toxin formation under field conditions should be more than 0.85 aw (Golob 2007). Relative humidity between 70 to 75% and moisture content between 14 to 15% may initiate growth of fungal species like *Aspergillus glaucus* on cereal grains, pulses, pelleted feeds and meals from oil seed (Williams 1991). Interactions may occur between environmental factors like temperature and water activity for fungal growth. It means that optimum temperature and water activity are interlinked and are not the same for some mycotoxins formation as for fungal growth. Optimum growth of fungal species like *F. verticillioides* was obtained at water activity of 0.995 aw and 20 and 25° C temperature range, but when value of water activity shifted to 0.98 aw, the optimum temperature changed between 30 to 35°C (Medina et al. 2017).

Implications of Mycotoxins in Different Livestock Animals

Food originated from animals and their products are consumed by the humans and may contain mycotoxin residues. However, in society, where consumers are main users, shifting of mycotoxin residues into milk and animal tissues from the feed should not be overlooked. The financial impact of mycotoxicosis in animals is many folds higher than mortality caused due to mycotoxicosis (Zain 2011).

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Table 1: Classification of toxigenic fungi (D'mello 2002; Santin 2005)

1.Field Fungi	2.Storage Fungi
1. Invade seeds of crop in the field.	Invade grain or seeds at storage.
2. High moisture content (20–21%).	low moisture content (13–18%).
3.These are mainly *Fusarium*, *Alternaria*, *Cladosporium* and *Diplodia*,	These are *Aspergillus* and *Penicillium*
3.Tropical and sub-tropical fungi	4.Temperate Fungi
1. Include *Asperigillus flaivus*, *A. parasiticuis* and *A. ochraceus*	Include *Penicillium expansum* and *P. verrucosum*.
2. Predominant under warm and humid conditions	More prevalent under cold conditions.

Table 2: Mycotoxin's prevalence in food and feedstuffs and their toxigenic effects (Jarvis 1971; Moss 1991; Grenier and Oswald 2011; Zaki et al. 2012; Milani 2018).

Fungal species	Mycotoxins produced	Food/feed substrate	Toxigenic effects	Biological activities
Aspergillus flavus, *A. parasiticus*	Aflatoxins	Wheat, maize, rice, peanuts, oilseeds, and cottonseed, milk and dairy products	Carcinogenic and immunosuppressive	Temp. 25-37°C Water activity. 0.94-0.99aw pH. Less than 6.0
Fusarium. Culomorum, *F. graminearum*, *F. sporoitrichioides*, *A. ochraceus*, *A. Alliaceus*, *A. melleus*, *A. ostianus*, *A. sulphureus*, *Penicillium*	Deoxynivalenol	Corn, wheat and their by-products	Acute temporary nausea, vomiting, diarrhea, abdominal pain, headache.	Temp. 23.75° C Water activity. 0.98aw pH. 4.91
virudicatum, *P. palitians*, *P. communne*, *P. variabile,*	Ochratoxin A	Wheat, barley, oats, cocoa beans, coffee beans, dried fruits, and wine	Carcinogenic and immunosuppressive and nephrotoxic	Temp. 30° C Water activity. 0.98aw pH. 4.0
F.acuiminatum, *F. equisetti*, *F. poae*, *F. semitictum,*	T-2 toxin	Rye, wheat, corn, barley and oat	Immunosuppressive	Temp. 10-37° C Water activity. 0.995-0.90aw pH. 7.3
F. culmoruim, *F. graminearuim*, *F. sambucinuim,*	Zearalenone	Maize, wheat, barley, rye and animal feed	Oestrogenic	Temp. 22-28° C Water activity. 0.98aw pH. 5.6-74
F. proliferatum, *F. verticillioides*	Fumonisins	Cereal grains	Carcinogenic and neurotoxic	Temp. 20-30° C Water activity. 0.94-0.99aw pH. 3-4
Fusarium *spp*. Myrothecium *spp*. Trichoderma *spp*. Trichothecium *spp*.	Trichothecene (type B)	Cereal grains and animal feed	Dermonecrotic	Temp. 30° C Water activity. 0.98aw pH. 4.0-5.0

Table 3: Production of mycotoxins in grains at optimum temperatures and water activity (Milani 2018)

Mycotoxin	Temperature (°C)	Water activity (aw)
Aflatoxin	30	0.99
Ochratoxin	25–30	0.98
Fumonisin	15–30	0.9–0.995
Zearalenone	25	0.96
Deoxynivalenol	26–30	0.995

Effects on Monogastric Animals

Monogastrics are more sensitive to toxic effects. Feed manufacturers should be aware that if feed analysis shows presence of even a single mycotoxin, it is most probable that other mycotoxins may also be present. Different fungal strains such as *A. flavus* and *A. parasiticus* cause aflatoxins (AFs) formation. AFs occur naturally in tropical and sub-tropical regions which include aflatoxin B1 (AFB1), aflatoxin B2, (AFB2), aflatoxin G1, (AFG1) and aflatoxin G2 (AFG2). AFB1 is the most predominant in feeds and very effective hepatocarcinogens. Reduced feed intake, reduced feed efficiency, growth retardation, decreased appetite, anorexia, depression, low milk production and immunosuppression are some effects of AFs. Under acute conditions (at higher level) mortality of animal cannot be ignored. AFM1 which is metabolized form of AFB1 can be transmitted to suckling piglets through milk (Prodanov-Radulovic et al. 2017).

Ochratoxin is produced by fungi of Aspergillus *species* in tropical and subtropical climates and in temperate region by Penicillium *species*. It exerts toxigenic effects on livestock and humans (Sherazi et al. 2015). Partially, in all animal species and particularly in monogastric animals, its principal target organs are kidneys. Ochratoxin A (OTA), which is a primary ochratoxin, has greater protein affinity due to which it accumulates in animal tissues (Mobashar et al. 2010).

Deoxynivaleinol (DON) is one of the most frequently mycotoxins detected in grains (Mishra et al. 2013). In fact, DON is also called vomitoxin because of its association with swine vomiting. DON is normally produced by *F. graminearum*. Animal gender, age and dose of DON are factors which determine sensitivity to DON toxicity (Hughes et al. 1999; Awuchi et al. 2021). Presence of relatively low microbial biomass and subsequently poor metabolism of DON in stomach of monogastric animals is the major explanation for their higher susceptibility towards DON (Maresca 2013).

T-2 is very toxic and is produced by *F. tricinctum*. Its primary targets in swine are immune system and GIT because of rapid cell division in these sites (Marin et al. 2013).

Zearalenone is one of the important mycotoxins of Fusurium *species* which is stable at storage and shows tolerance at high temperature during processing of food (Gajęcki 2002). In various studies, it has been shown that during feed processing, zearalenone was not further degraded and it was found in all animal feed products (Chang et al. 2017). Therefore, concentration of zearalenone in diet should not be higher than 0.25 mg/kg (Zinedine et al. 2007). Zearalenone has an affinity to react with estrogen receptors present in reproductive tissues and causes estrogenic effects (Gajęcki 2002).

Fumonisins (Fms) are primarily neurotoxic, but also have potential for carcinogenic effects. These are very hard to study as they are hydrophilic and not soluble in organic solvents. Fms. particularly FmB1 causes disruption of sphingolipid metabolism by intervention of sphingosine N-acyltransferase enzyme. In addition, these mycotoxins also affect liver function, protein metabolism and urea cycle in animal body (Richard 2007). Pigs are mostly affected by level of 0.2 mg FmB1/kg. In animal feed, the European Commission recommended maximum levels of Fms. Some text is deleted in maize is 0.06 mg/kg and it is 0.005 mg/kg for pigs. Fms. have a molecular structure similar to that of sphinganine (Sa) and sphingosine (So), which are components of sphingolipids and affect Sa/So ratio. An increased Sa/So ratio in serum from pigs fed FmB1 at concentrations of ≥5 mg/kg has been found (Galvano et al. 2005). It appears that below 100 mg FmB1/kg diet, pig performance is mostly affected (Santin 2005). Table 4 presents summary of permissible concentrations of mycotoxins in swine feeds (Gajęcki 2002; Battacone et al. 2010; Maresca 2013; Marin et al. 2013; Pereira et al. 2019).

Effects on Poultry Birds

Almost all poultry species are affected by aflatoxicosis. Continued feeding of aflatoxin contaminated diet at low level shows some harmful effects, however, mortality may occur at relatively higher contamination level (Ditta et al. 2018).

Comparatively, layer birds show greater resistance to aflatoxicosis than young birds; therefore, these birds it accepted some changes should not receive contaminated level above 0.05 mg/kg. Aflatoxins may cause reduced feed intake, reduced egg size, poor eggshell, lower egg production and fertility problem in layer hens. Since eggs are preferably consumed by humans and aflatoxins metabolites have been observed in egg yolks, therefore, feed manufacturers should find out another alternative energy source for these birds rather than corn which is more susceptible to aflatoxins. (Ditta et al. 2018).

T2-toxin is considered to be the most potent for poultry sector. It may induce some lesions in mouth, digestive tract, organs, and skin. Some other effects are low egg production, inhibition of protein, DNA and RNA synthesis (Akande et al. 2006). Usually, its contamination in feed is low; therefore, its immunosuppressive effects and secondary infections often make diagnosis difficult. During disease outbreak, if any modification in diet leads to health and subsequently better bird performance, this may be a clue toward mycotoxin poisoning (Sokolovi et al. 2008).

Poultry are rarely affected by acute effects of DON mycotoxin under normal conditions, but under field conditions, it can cause reduced feed consumption in layers and broiler breeders, which can be an indication of presence of T2-toxin. However, DON with 5 mg/kg in diet and even below this level can cause reduced production, poor immune system and greater susceptibility to infectious diseases (Murugesan et al. 2015).

Effects on Equine

Toxic effects of mycotoxins on horses are not well described due to unavailability of enough literature, however, under field conditions, their effects seem to be of greater significance. Several health problems like colic problem, neurological disorders, hypersensitivity, and brain lesions have been reported in equine as the mycotoxins related issues. Likewise, aggregate influence of mycotoxins present in diet at low levels may affect feed intake, growth rate, fertility and respiration rate in horses. Reduced feed intake is reported when horses are fed on a DON contaminated diet at 15 mg/kg (Raymond et al. 2003). North Carolina University reported 100, 31 and 44% of colic cases in horses fed with DON, T-2 toxin and zearalenone contaminated feed. Relationship between these mycotoxins and colic is not understandable which needs to be clarified (Gomaa et al. 2022).

Among Fumonisins, FmB1 at 10 mg/kg is well reported for leukoencephalomalacia in horses. A multifocal neurologic disease in a herd of horses has been observed related to Fms. Equine leukoencephalomalacia, also called moldy corn poisoning which affects horses in particular while mules occasionally and causes unstiffening of brain (Richard 2007). Increased level of enyzmes such as sorbitol dehydrogenase (SDH) and gamma glutamyl transferase (GGT), causing liver damage in horses, has been linked with AFB1 at 2 mg/kg of diet. However, some literature also reports death of horses due to consumption of AFB1 contaminated feed even at 0.3 mg/kg (Riet-Correa et al. 2013).

Horses are the simple stomach animals with a considerable microbial digestive ability present in the large intestine. Surprisingly, horses are more vulnerable to mycotoxicosis because in horse's major absorption of nutrients occurs prior to fermentation in small intestine compared to the ruminants where absorption occurs after microbial degradation in rumen (Riet-Correa et al. 2013).

Table 4: Maximum Level of mycotoxins incidence in swine feeds

Mycotoxin	Swine type	Max. tolerable level (mg/kg)	Toxic effects
Aflatoxins	Growing pigs Finishing pigs Breeding pigs	<0.001 <0.2 <0.1	Reduced feed intake, reduced feed efficiency, decreased appetite, anorexia, epistasis, low milk production, carcinogenic, immunosuppressant.
Ochratoxins	Pigs	<0.2	Nephrotoxic, hepatotoxic, immunotoxic , terategenic, depression, reduced feed intake, reduced body weight gain, diarrhea, polyuria, polydipsia, dehydration and high mortality
Zearalenone	Growing pigs Finishing pigs Breeding pigs	0.2 0.3 0.1 <0.5 in grain and grain products	Swollen vulivas, vaginal prolapses, enlarged uterus, shrunken ovaries, enlarged mammary glands and decreased fertility, variation in progesterone level and teratogenic responses in pigs
Deoxynivalenol (DON)	Pigs	<20% of the diet. < 0.001 in complete feeds	Decreased feed intake, reduced weight gain, vomiting, hemorrhagic skin, nausiea, diarrhea and gastrointestinal tract lesions
T-2 toxin	Pigs	< 0.001	Feed refusal, weight loss, poor cellular immune response, inhibiting protein synthesis and damage of DNA
Fumonisins	Pigs	>0.002	Reduced weight gain, reduced feed efficiency, damage to liver and pancreas, immune suppressive, reproductive difficulties, porcine pulmonary edema, hepatotoxic and nephrotoxic

Table 5: Tolerance levels of mycotoxins in ration (mg/kg) of horses

Mycotoxin	Horses	Source
Aflatoxin	0.01 mg/kg	(Ahmed et al. 2017)
Deoxynivaleol (DON)	2 mg/kg or 5 mg in grain feed and or should not be above 40% of diet	(Raymond et al. 2003)
Fumonisins	5 mg/kg as a maximum limit and or should not exceed 20% of the diet on DM basis.	(Riley et al. 1993)
Zearalenone	1 mg/kg had no effect on ovarian function	(Juhasz et al. 2000)
T-2 toxin	1 mg/kg diet had no effect on ovarian function	(Juhasz et al. 2000)

Horses at productive stage, late gestation, early lactation and those used for intensive work have high energy requirements which are generally fulfilled from grain feeding. Therefore, horses have greater exposure to mycotoxicosis in term of grain affinity. On the other hand, horses used for light work are generally kept on hay or forage. Under poor management conditions, these feeds are contaminated with molds and mycotoxins. Usually, moldy forages are less palatable than non-moldy forages and when horses are fed on these feeds, they refuse feed before ingesting enough feed to cause severe damage to intestinal tract and one of the typical examples in horses observed in such cases is mild colic (Gomaa et al. 2022).

Regrettably, due to greater palatability of grains even infected with molds would not affect feed intake of horses. As a result, horses are most frequently exposed to mycotoxins (Skelly 2015). Some tolerance levels of mycotoxins in ration of horses are given in Table 5.

Effects on Dairy and Beef Cattle

Mycotoxins, especially aflatoxin contaminated feed not only affects animal productivity and health, but it also impairs milk production. It is secreted into milk as aflatoxin M1 residues which account almost 1.7% of dietary level on an average. Considering the hazards of aflatoxin M1 residues in milk, dietary level of aflatoxin contamination should not be more than 0.025 mg/kg. Aflatoxicosis in dairy animals results in reduced feed intake, loss in body weight; less feed conversion ratio, reduced reproduction capacity, lameness, immunosuppression, hepatotoxicity, deleted nephrotoxicity and eventually may cause mortality in calves at higher contamination level. Considering different levels of AF in dairy feeds and consequences reported in various studies, chronic aflatoxicosis is a threat in dairy sector (Komboi et al. 2020).

In dairy animals, reduced feed intake, lower milk production, higher milk somatic cell counts, liver damage, impair rumen fermentation and reduced reproductive efficiency are some of the associated impacts of DON. Milk production is reduced when DON contamination is greater than 0.3 mg/kg (Charmley et al. 1993).

Generally, zearalenone is characterized by estrogenic effects in dairy cattle. Some common effects are low feed intake, reduced milk production, poor reproductive performance, and mammary gland enlargement. This mycotoxin can also cause abortions in dairy animals at higher contamination. deleted some text. Zearalenone can be a good marker for mycotoxin presence in feed and its level in feed should not exceed 0.25 mg/kg (Chang et al. 2017).

Main effects of T-2 toxin in dairy cattle are feed refusal, ataxia, gastroenteritis and reduced immune response in calves and reduced milk production in cows. At higher contamination, it causes death of animal. Therefore, a practical recommendation under best conditions is that contamination of T-2 toxin in ration of growing and lactating animals should not be more than 0.1 mg/kg. (Whitlow and Hagler 2010; EFSA et al. 2011).

Fumonosins are the most predominant in dairy animal feeds; therefore, their risks are very common to cattle. Dairy cattle are less sensitive to mycotoxicosis because of their higher potential for mycotoxin degradation in rumen. Cattle show 60 to 90% ruminal degradation of fumonisin. Nevertheless, when degree of mycotoxin susceptibility among dairy and beef cattle is compared, it appears that beef cattle are more resistant to mycotoxins due to variability in feed consumption and reduced stress factors like calving. Though, microbial degradation of mycotoxins in rumen might be a defensive factor for cows against acute toxicity. But even lower mycotoxin level may intermingle with other stressed factors to cause poor reproductive performance and subclinical losses. These losses have a greater economic impact than acute health problems but are more difficult to detect (Whitlow and Hagler 2010).

Beef cattle are also affected by aflatoxins, although complications are usually less critical as compared to monogastric animals Reduced growth rate in calves and rectal straining and prolapsed rectum are noticeable effects of aflatoxins at higher level (Agag 2004).

Since cattle in tropical and sub-tropical regions are usually fed on high roughage diets and they are fed on grain as a supplement. Therefore, aflatoxin contamination in grains at moderate level can occur. One can calculate proportions of grains in total ration based on 2.5% of BW and then acceptable level of aflatoxin in grain. This can be explained by an example of growing calves with BW of 272 kg will consume about 6.8 kg of total feed (272 kg multiplied by 2.5% equals 6.8 kg). In case of feeding 1.4 kg of grain plus forage to appetite, the grain will make up about 20% of total diet (1.4 kg divided by 6.8 kg equals 20%). In this case grain may contain up to 0.5 mg/kg of aflatoxin (0.1 mg/kg divided by 20% equals 0.5 mg /kg). Based on Thumb rule and personal expertise which is usually applied in calculation of mycotoxin level in grain portion of ration in tropical and subtropical zones. Gastroenteritis, intestinal hemorrhage, bloody faeces and immune suppression are T-2 toxin induced effects in beef cattle. While reduced feed intake, reduced meat production and diarrhea are important effects of DON. Toxic effects of zearalenone are vulvovaginitis, embryo mortality and cystic ovaries (Gajęcki 2002; Harčárová et al. 2020).

Economic Importance of Mycotoxins Intervention in Feed and Livestock Industry

Mycotoxins are more likely to influence almost 25% of the world's crops annually (Iheshiulor et al. 2011). In developed countries like US and Canada, estimated evaluating cost of mycotoxins is about $5 billion (Madhysatha and Marquardt 2019). Most of the foods and feeds are lost during manufacturing and uncontrolled storage conditions in developing countries which, however, can be consumed or available for trade purposes (Aidoo 1991). One of the major sources of economic losses and greater management cost in animal husbandry is mycotoxins in animal feed (Adamse et al. 2012).

Mycotoxin contamination of different feed products cause noteworthy economic issues at all trading stages. Therefore, without following the Codex Alimentarius Commission's guidelines for risk assessment and acceptable procedures, countries dealing with import of these products have restrictions and deleted some text have adverse impacts on economy of these, deleting some text countries (Baht and Vashanti 1999). Considering level of mycotoxin toxicity and its prevalence in each country, mycotoxins regulatory limits for animal feedstuffs are variable and even in some countries, there are no guidelines (Charmley and Trenholm 2021).

Nutritional worth of forages and most of the cereal crops, and subsequently animal productivity are badly affected by mycotoxicosis (Iheshiulor et al. 2011). When fungal infestation occurs in crops, nutrients are utilized for fungal growth. This affects animal feed intake due to reduced nutrients and energy content in particular and therefore, decreased animal productivity (Golob 2007). The economic losses due to mycotoxicosis include crop losses, loss of animal and human life, expenses of increased health care, and veterinary care, low animal productivity, disposal of contaminated foods and feeds, and cost on mycotoxins regulatory programs for control measures (Zain 2011).

Risk Management: Prevention of Mycotoxins in Feeds

Considering toxic effects and high economic losses due to mycotoxins, different risk management strategies have been established which mainly include.

1. Prevention of mycotoxin contamination.
2. Degradation and/or detoxification of mycotoxins
3. Inhibition of mycotoxin absorption in the gastrointestinal tract (Santos et al. 2010).

Pre and Post-harvest Preventive Measures

Fungal contamination and mycotoxins productions in the crops usually occur at pre and post-harvest stages (Omotayo et al. 2019). Control of pre-harvest mycotoxin contamination is getting more attention globally and some advanced techniques are being applied for this purpose. Breeding resistant crops, established agronomic practices to prevent plant stress, deleted and reduced soil fungi forming mycotoxins and harvesting at the optimum stage of maturity are some of the important mitigation measures for mycotoxicosis during pre-harvest stage (Xu et al. 2022).

Mitigation of kernels from insect infestation is one of the vital strategies to control propagation of *A. flavus* and *A. parasiticus* at pre-harvest stage (Lopez-garcia et al. 1999). Optimum stage of maturity for crop harvest is very important for later use and good quality product (Aidoo 1991). Field heat causes rapid deterioration, fungal colonization and mycotoxin formation; therefore, it is very necessary to harvest crop early during the day at sunset time. Mechanical damage and delayed harvest favor mycotoxin contamination (Peraica et al. 2002; Kabak et al. 2007).

Reducing moisture content before storage is one of the simple and most economical means of safe storage. Therefore, grains must be dried by natural conditions before storage. Mycotoxin problems in silage occur only through oxygen exposure, when it is not tightly covered. It is very necessary to store feedstuffs at suitable temperature and humidity. It is commonly observed that the spoiled product at pre-harvest stage contains deleted a significant mycotoxins load at storage. Therefore, removing and/or reducing toxic effects in the food and animal feeds, physical, chemical and biological detoxification strategies are very necessary (Pemberton and Simpson 1991).

Physical Strategies

Some of the important physical techniques for reducing or inactivating mycotoxins in feeds are cooking, boiling, baking, frying, roasting and extrusion (Aiko and Mehta 2015). These procedures eradicate mycotoxins from contaminated grain products and their presence in the gastrointestinal system (He and Zhou 2010). Mycotoxins in feedstuffs can be degraded through heat treatment; however, this may destroy vitamins and denature proteins (Samarajeewa 1991).

Mycotoxins in various feeds can be extracted using solvents. The most common solvents for this purpose are ethanol (95%), aqueous acetone (90%), isopropanol (80%), hexane-methanol, methanol-water, acetonitrile-water, hexane-ethanol-water, and acetone-hexane (Kabak et al. 2007). However, this approach cannot be used on large-scale due to its high cost and issues of disposal of toxic extract (Rustom 1997).

Bioavailability of mycotoxins can be reduced through incorporation of adsorbents into feeds which reduce mycotoxin uptake and transport to target organs. These are substances with high molecular weight having binding ability for mycotoxins in GIT. Adsorbents inhibiting mycotoxin absorption are excreted from body through faeces as adsorbent-toxin complex (Santos et al. 2010). These do not dissociate in GIT and prevent animals against mycotoxin exposure. Carbon based organic polymers and silica based inorganic compound are important adsorbing agents. Most commonly used inorganic adsorbents are natural clay products, and synthetic polymers (Huwig et al. 2001; Stoev 2013). Organic binders have been highly proposed, because of their effectiveness against a wide range of mycotoxins. These are decomposable and therefore will not contaminate the environment after being excreted from animals. While inorganic binders are integrated at high frequency as these are collected in animal manure and then spread in the field and cause contamination to environment (Jouany 2007).

Chemical Methods

Some chemicals are very effective against reduction, destruction, and/or inactivation of mycotoxins like acids, bases, oxidizing agents and reducing and chlorinating compounds. Although most of these chemicals are effective against mycotoxins, but chemical detoxification of mycotoxins is not appropriate because these often reduce nutritional value of chemically treated feeds (Abdel-wahhab and Kholif 2008).

Biological Detoxification of Mycotoxins

Biological detoxification of mycotoxins means application of microbes, their enzymes and metabolites for binding and degradation of mycotoxins. The microbiota in application should be safe, nonpathogenic, having degradation potential, free of improper odor or taste and preserve nutrients in food (Varga et al. 2010). A diversity of bacteria, yeasts, and molds has mycotoxin degradation potential. The clue behind this is using enzymes precisely for breakdown of a single mycotoxin or group of mycotoxins into a non-toxic molecule (Ji et al. 2016).

Probiotics are live microorganisms which after incorporation in foods, drugs and animal diets in sufficient quantities exert positive effects on the host health. Probiotic bacteria like *Lactobacillus* strains (LAB) have a very promising mycotoxin detoxification potential. LAB bacteria cause detoxification of AFB1. An assessment on five *Lactobacillus* strains including *L. rhamnosus* GG, *L. rhamnosus* LC705, *L. acidophilus*, *L. gasseri* and *L. casei* shows that these strains have detoxification efficacy up to 80% (Haskard et al. 2000) and *L. rhamnosus* GG causes 70% disappearance of ZEN (El-Nezami et al. 1998). Similarly, *L. acidophilus* strain (VM 20) can remove OTA by 90% (Fuchs 2008).

Some actino-bacterial strains in soil have mycotoxin binding ability and detoxification potential. AT8 strain can effectively detoxify OTA up to 52.61%. deleted and *Streptomyces spp*. and *Aspergillus flavus* (NRRL 62477) in combination can also remove AFB1 and AFB2 in vitro up to 73%. *Lysinibacillus ssp*. strain isolated from chicken large intestine digesta has greater removal ability of ZEN mycotoxin (Nahle et al. 2022).

S. cerevisiae, a yeast strain used in food industries has ability to degrade and detoxify OTA and AFB1 and can decrease AFM1 contents in milk (Piotrowska and Zokwska 2005).

Some fungal species of Aspergillus can convert AFB1 to AFB2 through various enzymes (Ji et al. 2016). White rot fungi can degrade AFs up to 87% through laccase enzyme (Alberts et al. 2009) while *A. niger* (ND-1) up to 58.2% (Zhang et al. 2014) through laccase and Mn-peroxidase (Singh and Mehta 2022).

Conclusion

Mycotoxins are toxic secondary metabolites of toxigenic fungi. Mycotoxin contaminated feeds affect animal health and productivity. Contamination of feeds may occur during pre- and post-harvests. Huge economic losses occur annually in the feed and animal sectors. Consequently, some measures are needed to eliminate and/or deactivate mycotoxins in feedstuffs. Various preventive measures for mycotoxin production and its drawbacks are being applied globally. These decontamination measures include biological, chemical and physical techniques. Nevertheless, physical and chemical methods have inadequate efficacy, safety concerns, palatability losses, and are costly. They have also been condemned due to loss in nutritive value of the feeds, and negative effects on animal and human health. Using of adsorbents and microorganisms/enzymes may be more appropriate. Further research is warranted to figure out potential of biological methods at molecular level in comparison with other decontamination procedures.

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Antifungal Potency of Green-Fabricated Nanoparticles-An Overview of Recent Advances

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INTRODUCTION

Fungi are eukaryotic unicellular or multi-cellular organisms found in all types of environments across the world. They have various shapes and sizes, ranging from the size that can be seen with the naked eye, like mushrooms, to tiny yeasts and molds. Several hundred fungi cause fungal infections or diseases, even though the majority of fungi do not significantly contribute to human diseases (McKeny et al. 2021).

Invasive fungal diseases (IFDs), which collectively affect more than a billion people worldwide, are common diseases caused by fungi that range from allergic syndromes to superficial, and debilitating diseases that may be fatal (Bongomin et al. 2017). Invasive fungal infections severely threaten public health and are an underappreciated aspect of the global antimicrobial resistance crisis. Pathogenic fungi that infect people are developing resistance to all approved systemic antifungal medications during a time of significant environmental change worldwide and growing at-risk populations (Fisher et al. 2022).

Opportunistic fungal pathogens are frequently found in our close environments, and many have a high spore production capacity. As a result, various environmental fungal pathogens are regularly inhaled by humans as bioaerosols. Those with weakened health or immunity are more susceptible to several illnesses, including superficial, allergic, chronic, and life-threatening IFDs. At the same time, most environmental fungi do not manifest any observable pathophysiological events in healthy people. The number of patients at risk for IFDs is rising, and older people are among those (Fisher et al. 2022). Indoor and outdoor environments are both favourable to fungi, according to the statement "Risk increases synchronously with a time of exposure," however, indoor airborne fungi are more effective on human health than outdoor airborne fungi (Omolola et al. 2018; Al-Bader et al. 2021).

Aspergillus species., *Candida* spp., involving *C. auris*, and *Mucoromycota* spp., which display strong innate and acquired resistance to antifungal therapies, have increased their prevalence in patients with severe viral infections for instance the influenza virus and COVID-19 patients (Singh et al. 2016; Arastehfar et al. 2020; Janssen et al. 2021). The vulvovaginal candidiasis is also more prevalent among women (Shnawa et al., 2018). Invasive infections brought on by filamentous fungi have posed a significant threat to public health over the past few years on a global scale. Human infections can be acquired by *Aspergillus*, *Coccidioides*, *Mucorales* (the most prevalent filamentous fungi), and *C. auris* (a non-filamentous fungus). They can cause life-threatening illnesses in immunosuppressed people, HIV/AIDS patients, people with uncontrolled diabetes, people with haematological diseases, people undergoing chemotherapy, and transplant recipients (León-Buitimea et al. 2021).

Just four classes of systemically acting antifungal medications have historically dominated. The treatment includes polyenes, azoles, echinocandins, and the pyrimidine analogue 5-flucytosine (Robbins et al. 2017). They all have disadvantages regarding pharmacokinetics, pharmacodynamics, resistance mechanisms, and the compounds' toxicity, in addition to their range of activity limitations. Additionally, there are some restrictions on clinical efficacy and efficiency, primarily due to their physical and chemical characteristics, such as their hydrophobic nature, which results in low solubility in water, and selectivity issues resulting from the similarities between fungi and human cells (Chang et al. 2017; Souza et al. 2017). Moreover, Traditional systemic mycosis antifungal therapy is expensive, frequently limited, and has significant toxic side effects. Nanotechnology has emerged as a novel strategy to increase the effectiveness of conventional antifungal medications and enhance the activity of antifungal drugs. It enables lower toxicity, better biodistribution, drug targeting and promising in vitro and in vivo outcomes (Souza et al. 2017). Nanoparticles are considered significant alternatives to help in drug delivery (Renzi et al. 2021). Using these NPs in conjunction with antifungals has been shown to raise solubility, improve permeability, and bioavailability, increase storage stability, prolong half-life, reduce therapeutic cost, and ensure proper drug dosage (Faustino et al. 2020). Although, finding new antifungal drugs faces a

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particular challenge due to the rising incidence of fungal diseases resistant to treatment. Recently, nanotechnology applications have been included among the most promising areas of study to address these issues. The ability of nanoparticles to target specific locations where fungi are found, their capacity to improve the pharmacological effects of medications, and their potential to optimize their physiochemical properties have all been mentioned as reasons why they represent possible solutions (Sousa et al. 2020).

Nanobiotechnology

Nano-biotechnology is a division of nanotechnology that offers NPs production for specific applications with little risk of bio-systems resource influences. " Nanobiotechnology" refers to a broad field that includes the production and subsequent use of particles smaller than 100 nm (Ahmad et al. 2003; Shnawa et al. 2021 a).

Recent studies have concentrated on developing practical, eco-friendly green methods for generating metal NPs.The Green production of metal NPsis easy, clean, effective, safe and non-expensive as they use bio-resources (plants, fungi, algae, and microorganisms) that can serve as reducing and stabilizing agents. Currently, NPs of some metals or metal oxides are used as a medication to treat different diseases and enhance human health due to their antimicrobial action (Lee et al. 2011; Shnawa 2018). Plants, algae, filamentous fungi, yeast, bacteria, and viruses are a few examples of the various organisms that could be considered sources for NPs. Some scientists looked into using microorganisms to make NPS out of eco-friendly materials like cadmium, gold sulphide, and silver (Ahmad et al. 2016; Mukherjee et al. 2001). Bacteria, Actinomycetes, and fungi were applied to make NPs (Singh et al. 2016). The biosynthesis of nanoparticles has certain advantages over other techniques; 1- phytochemicals found in plant extracts will reduce, cap, and stabilize the nanoparticles. 2- these phytochemicals can be produced in a single pot and are non-toxic, inexpensive, and environmentally friendly. 3- some OH and other functional groups continue to bind with the nanoparticles even after the separation and purification stages of NP preparation, making the nanomaterials more reactive than those made using other techniques (Raj et al. 2018; Shnawa et al. 2021 b) as shown in Fig. 1.

Nowadays, under experimental and greenhouse circumstances, the fungicidal activity of MgO nanoparticles (nMgO) is assessed against soilborne *Phytophthora nicotianae* and *Thielaviopsis basicola*. According to in vitro research, nMgO is more effective than macroscale equivalents at suppressing sporangium development, spore germination, and fungal growth. It was discovered that there were direct contact interactions between nanoparticles and fungal cells or that they adhered to them, which led to changes in cell morphology (Chen et al. 2020). Depending to Jamdagni et al. (2018) study, green-synthesized ZnO nanoparticles (NPs) demonstrated effective antifungal activity. They found that *Aspergillus niger* was the most sensitive among the tested fungi. Furthermore, extensive studies have used nanoparticles to treat mycoses, especially corneal and invasive infections (Balabathula et al. 2020; Faustino et al. 2020). Previously, Xia et al. (2016) demonstrated the effectiveness of silver nanoparticles by showing antifungal properties against the pathogenic and medication-resistant fungus *Trichosporon asahii*.

Regarding the biocompatibility of the green synthesized nanoparticles, Jalil et al. (2021) showed no noticeable toxic effect of Ag NPs at low concentrations on RBCs. They emphasized that the green fabricated AgNPs are biocompatible and can be used as safe agents at low concentrations. Another recent finding concluded that the bio-synthesis ZnO-NPs from *Z. spina-christi* leaf were biocompatible at the tested experimental levels (Shnawa et al. 2022). Some significant recent articles summarizing the biosynthesis of different nanoparticles with their antifungal potency are shown in Table 1.

Mechanisms of Action of Nanoparticles against Fungi

Regarding antifungal compounds, it's crucial to consider the nanoparticles' site of action (on the cell membrane, cell wall, DNA, or RNA), as well as their surface properties. The portion of the cell that communicates with the outside world is the fungal wall, which regulates cell permeability. ZnO nanoparticles caused the cytoplasmic contents to liquefy, which reduced the cytoplasm's electron density and resulted in a noticeable separation of the fungal cell wall (Arciniegas-Grijalba et al. 2017). Moreover, Gunalan et al. (2012) concluded that compared to chemical ZnO nanoparticles and other common antimicrobials, biologically synthesized ZnO had a higher growth inhibition of fungi and bacteria. Smaller particles' increased bioactivity is attributed to their high surface area-to-volume ratio. According to Jamdagni et al. (2018), Ag-NPs significantly disrupt the target microorganisms' cell walls and plasma membranes, causing pores to form and electrolyte imbalances. The broken membrane allows fungicides to enter cells more effectively (Khatami et al. 2018).

Additionally, these ZnO-NPs demonstrated antifungal activity against the pathogenic fungus *Erythricium salmonicolor*, which causes the coffee crop disease, which is also known as the pink disease (Arciniegas-Grijalba et al. 2017).

Erazo et al. (2019) further concluded that reactive oxygen species are produced due to the electronic properties of the semiconductor material and the ZnO-NPs' capacity to obstruct electron transfer in biological systems. The ZnO-NPs' surface reaction with water produces hydroxyl radical (OH) and hydrogen peroxide H_2O_2.

The fungal cell wall significantly contains chitin and chitosan. They have more than 7% nitrogen (N) content. Additionally, they have a regular distribution of free amino groups, which particular acids can positively charge them by protonation and giving them a polycation performance.

Table 1: Antifungal potency of nanoparticles fabricated from bio sources.

Nanoparticles	Green sources	Antifungal potency	References
Selenium (SeNPs)	*Halomonas elongate* bacterium	Antifungal properties against *C. albicans*.	(Safaei et al. 2022)
Selenium (SeNPs)	Green synthesis	Antifungal properties of nanoparticles (SeNPs) against *C. albicans* and *C. glabrata*	(Hosseini Bafghi et al. 2022)
Selenium (Se NPs)	Fabricated by Bacillus species Msh-1	Against *A. fumigatus* and *C. albicans*. They demonstrated that mould cells were less sensitive than yeast cells.	(Shakibaie et al. 2015)
Silver (AGNPs)	Green production of AgNPs using ribose as a reducing agent	They demonstrated high action against *C. albicans* and *C. tropicalis*	(Mallmann et al. 2015)
Silver (AgNPs)	Silver nanoparticles (AgNPs) were synthesized by *Ligustrum lucidum* leaf extract.	The antifungal properties particularly notable against the phytopathogen *Setosphaeria turcica*	(Huang et al. 2020)
Silver (AgNPs)	Green biosynthesized	*A. fumigates*, *A. niger*, *A. flavus*, *Trichophyton rubrum*, *C. albicans*, and Penicillium species have all displayed growth inhibition by AgNPs.	(Mansoor et al. 2021)
Zinc oxide (ZnO NPs)	Aloe leaf broth extract	Revealed activity against fungal strains *A. flavus*, *A. nidulans*, *Trichoderma harzianum*, and *Rhizopus stolonifer*	(Gunalan et al. 2012)
Zinz oxide (ZnO NPs)	Flower extract of *Nyctanthes arbor-tristis*	*Active against Alternaria alternata , Aspergillus niger , Botrytis cinerea* (ITCC 6192), *Fusarium oxysporum* and *Penicillium expansum*	(Jamdagni et al. 2018)
Silver (AgNPs)	Waste-grass-mediated green synthesis	*Fusarium solani and Rhizoctonia solani*. The highest effect of AgNPs against *F. solani* was 90% at a concentration of 20 µg/ml of AgNPs.	(Khatami et al. 2018)
Gold (Au NPs)	AuNPs produced from *Garcinia kola* leaf	Activity against *Fusarium oxysperium* was greater than *C. albican*, and *Penicillium camemeri* was greater than *A. flaws*	(Adekunle et al. 2020)
Gold (AuNPs)	AuNPs biosynthesized by mycelial biomass of endophytic fungus *Phoma* sp.	Antifungal activity against *R. solani* (the rice fungal pathogen).	(Soltani Nejad et al. 2022)
Silver (AgNPs)	Biofabricated AgNPs using cell-free filtrate of *Trichoderma viride*	Compared to chemically fabricated Ag, biosynthesized Ag NPs improved the reduction in dry weight of *Fusarium oxysporum* and *Alternaria brassicicola* by 20% and 48.8%, respectively.	(Kumari et al. 2019)
Ag-Cu NPs	Ag-Cu NPs from bark extracts of *Garcina kola*	Showed antifungal activity and ordered as *A. flavus* > *Candida albicans* > *Fusarium oxysperium*> *Candida tropicalis*	(Akintelu and Folorunso 2019)

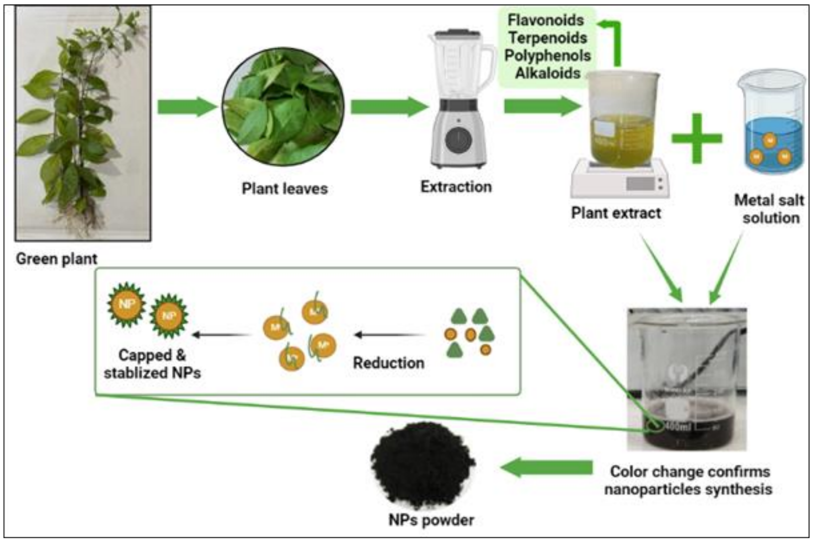

Fig. 1: General mechanism for green synthesis of nanoparticles. Phenols, flavonoids and others are secondary metabolites of plants that have been reported to act as bio-reductants of metallic ions in an aqueous environment. The functional groups in these plant components serve as reducing, capping and stabilizing agents for nanoparticle synthesis

This led to, lipids, proteins, dyes, and other negatively charged substances can all be bound to chitin. As a result, these characteristics would also make the chitosan vulnerable to attack by reactive oxygen species (ROS) (Erazo et al. 2019).

It is shown that various metal nanoparticles, for instance, Ag-NPs, ZnO-NPs, and CuO-NPs, as well as their ions (Ag+, Zn2+, and Cu2+), are toxic to fungi. The toxicity mechanism against fungi varies depending on the metal nanoparticles' physicochemical properties, the concentration used, and the soil environment (Ameen et al. 2021).

A study examined the antifungal activity of AgNPs and their mechanisms against the fungicide-resistant and fungicide-sensitive strains of *F. graminearum*. They also clarified their effects on the production of mycotoxin deoxynivalenol (DON) and evaluated the potential of AgNPs for the management of *Fusarium* head blight (FHB) disease in cereal crops.

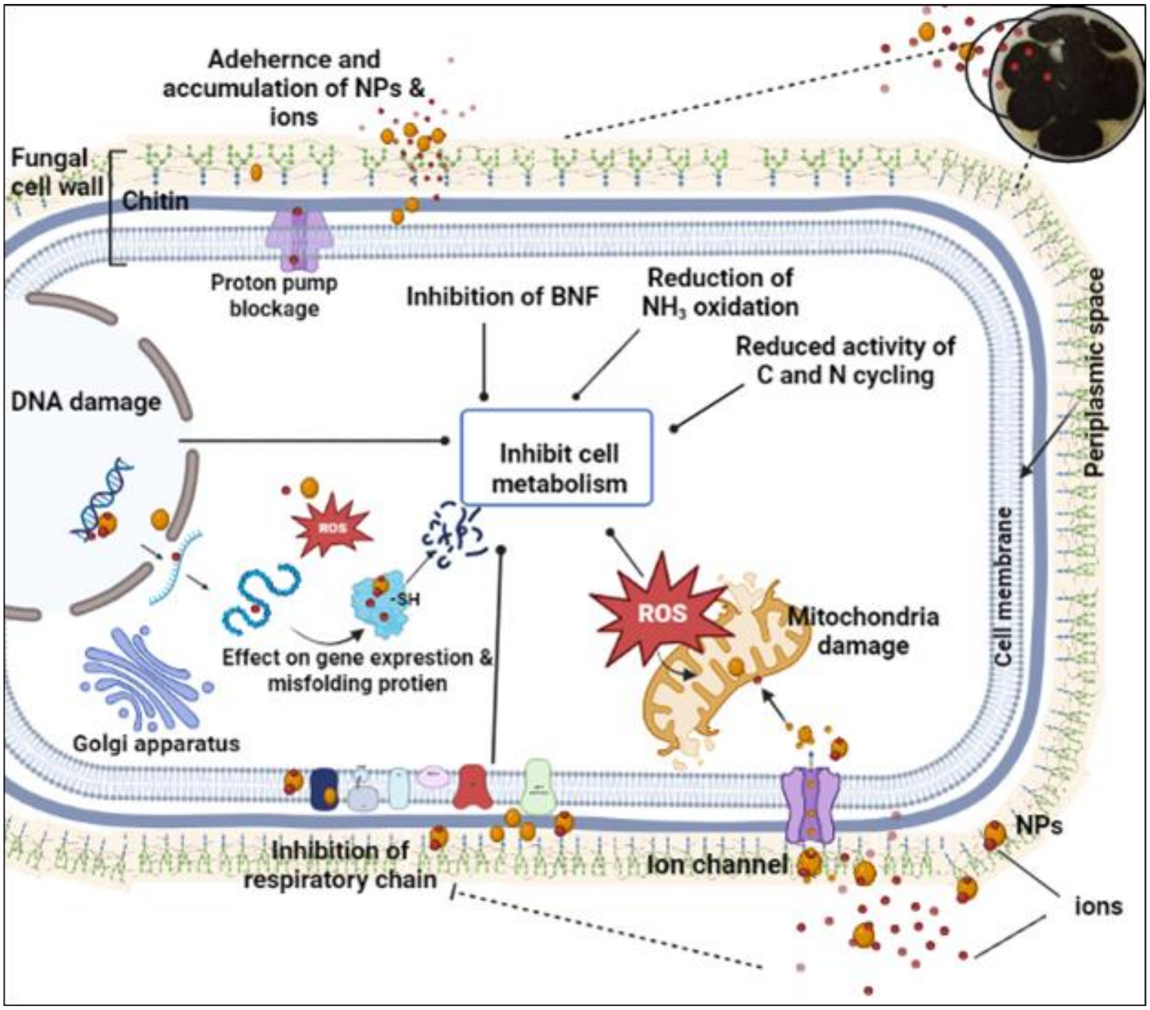

Fig.2: Proposed mechanisms for antifungal activity of NPs. The NPs are introduced into the cell through proton pumps and enhance reactive oxygen species, causing harm to the cytoplasmic organelles, inhibiting proton pumps, damaging the cell wall, and targeting the metabolic process. ROS: Reactive oxygen species, NPs: Nanoparticles, and BNF: Biological nitrogen fixation.

Silver nanoparticles (AgNPs) exhibit excellent antifungal activity against both fungicide-sensitive and -fungicide-resistant *F. graminearum* strains through several mechanisms, including disruption of fungal development and cell membranes as well as disruption of cellular energy utilisation and metabolism pathways. Even though AgNPs may upregulate azole resistance-related ATP-binding cassette (ABC) transporter genes, the control efficacy of the fungicides was unaffected. But each coin has double sides: AgNPs have potent antifungal action against *F. graminearum*. However, these can also be able to lead to increased production of the infamous mycotoxin deoxynivalenol (DON). AgNPs must be utilized as efficient and alternative medicinal candidates for pathogens that produce mycotoxin by balancing their antifungal action and DON fabrication. Combining AgNPs and DON-reducing fungicides may be an option for managing FHB disease. Future research is required to assess AgNPs' antimicrobial potential in practical agricultural settings (Jian et al. 2022).

To overcome fungi resistance, AgNPs are used. AgNPs disrupt cell walls, damage surface proteins, harm nucleic acids by generating and accumulating ROS and free radicals, and obstruct proton pumps. According to Du et al. (2012), AgNPs cause the accumulation of silver ions, which interferes with intracellular ion efflux and inhibits respiration, causing harm to the electron transport system. The antifungal activity of nanoparticles is attributed to their smaller size to large surface ratio. AgNPs that are smaller in size can easily cross cell boundaries. AgNPs' toxicity is partially explained by the generation of ROS, which triggers apoptosis. According to various theories (Beer et al. 2012; Kim and Ryu 2013), the in vitro toxicity of AgNPs is caused by either the interaction of Ag ions and AgNPs or their effects. Further research is required to determine the precise mechanisms and modes of action of AgNPs (Mansoor et al. 2021). The proposed mechanisms of NPs action against fungi are illustrated in Fig. 2.

Antifungal Drugs and Nanoparticles for Antifungal Drug Delivery

The main variety of antifungal drugs used in mycoses therapy contains polyenes (amphotericin B, hamycin, natamycin, and nystatin), azoles (imidazoles such as ketoconazole, miconazole, etc.), triazoles (fluconazole, itraconazole, ravuconazole, posaconazole, etc.), thiazole (abafungin), pyrimidine analogue (flucytosine), squalene monooxygenase (allylamines (naftifine, and terbinafine), benzylamine (butenafine), and mitotic inhibitor (griseofulvin) (Campoy et al. 2017; Nami et al. 2019). Nanoparticles are particularly used to treat fungal infections. Because of their unique properties, these particles—unlike pure antibiotics—can exert a more substantial inhibitory power even when used in lower concentrations than pharmaceuticals. Antifungal drugs have been used in delivery systems despite their poor pharmacokinetics, decreased efficacy, limited tissue penetration, poor aqueous solubility, decreased bioavailability, side effects, and drug stability. These factors are significant and well-known (Soliman 2017; Hassanpour et al. 2020).

New therapeutic options for treating aspergillosis and infections caused by *Candida* have been developed using various strategies, including incorporating coating materials, complexes made through green chemistry, or combining polymers. However, there are few treatment options for coccidioidomycosis and mucormycosis, and the majority of them are still in the research and development stage (León-Buitimea et al. 2021). Hence, more studies are needed to explore novel therapeutic opportunities that solve this problem. Nanoparticles can overcome several unfavourable drug characteristics of flexibility, multifunctionality, and broad properties (Nami et al. 2021).

Various NPs subtypes are used as antifungal delivery systems, which include:

1-Solid lipid Nanoparticle

The characteristics of the drugs loaded in such a formula depend on the components used and the characteristics of the drugs. Real problems are observed in the cases of cutaneous infections treated by traditional systemic medicines. The first is poor medication accessibility at the infection site and the second is the drug toxicity and side effect of drugs. The new drugs are characterized by continued release, biocompatibility, and decreasing side effects (Pople et al. 2006; Trombino et al. 2016).

2- Nanostructured Lipid Carrier

In this application, solid and liquid lipids are composed of an essential matrix (Aghebati-Maleki et al. 2020). The solubility of the compound increase when mixed with antifungals. The shape of molecules is also betters encapsulated, and they have a high affinity to bind with cell receptors; thus, they are absorbed faster. Finally, using NLCs as drug carriers expands the drug's effective period in the body by protecting the composition from biodegradation (Haider et al. 2020). Amphotericin-B was loaded in such a composition and gave a better result in treating keratitis (Fu et al. 2017).

3- Cubic Liquid Crystalline Nanoparticle

This structure, also known as cubosomes, is a discrete nanostructured particle characterized by its bio-adhesive and biocompatibility properties (Spicer 2005). Several properties were observed for cubosomes when they were used as drug carriers, and they possess a small size with minimal viscosity and larger interfacial areas. The core of the molecule is hydrophobic. The structural properties result in efficient drug loading (Yang et al. 2012; Zhang et al. 2020).

4- Silver Nanoparticles

Silver nanoparticles (AgNPs) have been widely used in medicine. The particles have distinctive chemical and physical characteristics. They possess thermal, high electrical conductivity and biological properties (Nozari et al. 2012; Zhang et al. 2016). Studies showed that the combination (amphotericin-B /silver nanoparticles) has high antifungal activity against *Candida, Aspergillus,* and *Fusarium* than the pure drug (Ahmad et al. 2016; Tutaj et al. 2016).

5- Polylactic-co-glycolic Acid Nanoparticle

For the past 20 years, polylactic-co-glycolic acid (PLGA) is one of the greatest alluring polymeric candidates used to create devices for tissue engineering and drug delivery. In addition to having a wide range of erosion times, tunable mechanical properties, and of utmost importance, being an FDA-approved polymer, PLGA is also biocompatible and biodegradable. In particular, PLGA has received extensive research attention for creating devices for the controlled delivery of small-molecule medications, proteins, and other macromolecules in both commercial and academic settings (Makadia et al. 2011).

6- Gelatin as a Natural, Adaptable Biopolymer

Collagen is hydrolyzed to produce the protein known as gelatin. For use in nanobiotechnology and nanopharmaceuticals, gelatin is a desirable biodegradable material. Due to their biocompatibility and biodegradability, gelatin nanoparticles (NPs) have been widely used as a drug and gene carrier to target sick tissues, including cancer, tuberculosis, HIV infection, and the treatment of vasospasm and restenosis (Yasmin et al. 2017). In a 2017 study on managing infection and inflammation in keratitis, Ahsan and

Rao created a double-desolvation ketoconazole-encapsulated gelatin nanoparticle. They discovered that these NPs could lengthen drug residence time, reduce inflammatory cytokines, and release antifungal drugs in their study on *Aspergillus flavus*- infected corneas of rats (Ahsan and Rao 2017).

Conclusion

Due to their rising annual incidences and mortality rates, fungal infections are becoming more and more critical in today's world. The majority of commercially available antifungals are successful in treating these fungi, but due to problems with tissue penetration, aqueous solubility, reduced bioavailability, drug efficacy and resistance, side effects, and drug pharmacokinetics, researchers have been looking for ways to enhance the effectiveness of the currently available medications. In order to maximize the effectiveness of antifungals over the past 20 years, the use of NPs drug-delivery systems to broaden the scope of antifungal functions has become increasingly important. Consequently, more study is needed to develop promising therapeutic opportunities that lead to advancement in this field.

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